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RESEARCH PAPER

Comparative Study of Three Methods of Sample Preparation for Proteomics Research

SUN Ning-Ning[1], WANG Zi-Jian[1], GAO Xiang[1], CHENG Xin[1], SUN Wan-Chun[2], YANG Jing-Bo[1], LIU Ning[1,*]

[1] Central Laboratory, The Second Hospital of Jilin University, Changchun 130041, China
[2] Key Laboratory of Zoonosis, Ministry of Education, Jilin University, Changchun 130062, China

Abstract: Protein sample preparation is a vital issue for proteomics research. Due to different physical and chemical properties of reagents used for sample preparation, the ability of different reagents to disrupt cell or tissue, as well as to solubilize a variety of proteins, is very different. In the present study, three sample preparation methods widely used in proteomics researches (Triton X-100 method, urea method and TRIzol method) were compared by using mass spectrometric method in analyzing proteins isolated from cultured 293T cells, followed by bioinformatics analyses. The results indicated that the number of the identified proteins extracted by Triton X-100 method was almost the same as that by urea method, whereas the number of the identified proteins extracted by TRIzol method was approximately 8 percent smaller than those by either Triton X-100 or urea method. A large difference was found among the protein categories identified by three extraction methods, and only 32 percent proteins were identified from samples by all three methods. The profiles of proteins prepared by three methods were compared and further analyzed using functional classification software. This study provides a rapid, effective and comprehensive tool for evaluating the sample preparation methods for proteomics study.

Key Words: Proteome; Sample preparation; Urea; Triton X-100; TRIzol; Liquid chromatography-tandem mass spectrometry

1 Introduction

In proteomics study, sample preparation is one of the primary issues. Different sample preparation methods may lead to different profiles of proteins extracted from specific cells or tissues[1–3]. At present, a variety of sample preparation methods have been applied to proteomics research. A typical method for protein preparation is to use the high concentration solution of urea with trihydroxymethyl aminomethane (Tris) (pH 8.5), thiourea and CHAPS and so on as the solution system, which have been widely used in two-dimensional electrophoresis[4–6]. Another lysis buffer contains the surfactant Triton X-100, the main reagent in several commercially available kits for cell lysis, such as a product (# 9803) from Cell Signaling Technology[7]. Triton X-100 is a non-ionic surfactant, and 1% (*V/V*) Triton X-100 can disrupt cell membrane even under non-denaturing conditions. Therefore, this kind of lysis buffer can maintain native conformation of protein[8,9]. In addition to these two commonly used lysis buffers, other types of lysis buffer have been reported to be used for sample preparation in proteomics research[10–12]. Among them, a promising method uses TRIzol to disrupt cells and tissues[13,14], which is commonly used to extract total RNA[15]. RNA, DNA and protein can be individually precipitated by chloroform, alcohol and isopropyl alcohol, respectively, after TRIzol reagent is added into cells or tissues. Because TRIzol inhibits a variety of endogenous enzymes, RNA, DNA and protein samples of high quality can

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* Corresponding author. Email: liu_ning@jlu.edu.cn
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be obtained. TRIzol is more suitable for the preparation of biological samples containing virulent and contagious bacteria or viruses (e.g. avian influenza viruses).

Until now, there has been no method by which the whole proteome in cells or tissues can be completely extracted due to the diversity and complexity of proteins in organisms. Due to different physical and chemical properties of reagents used for sample preparation, the ability of different reagents to disrupt cell or tissue, as well as to solubilize a variety of proteins, is very different. Therefore, researchers have to choose one of the most suitable methods for protein extraction based on the research target and research purpose. However, the differences in the abilities to lyze cells and to dissolve proteins between different methods for sample preparation are not very clear. Some researchers have utilized two-dimensional electrophoresis (2-DE) technology to analyze and compare the difference between different sample preparation methods[16]. 2-DE technology can separate a mass of proteins in one experiment depending on the isoelectric point (pI) and molecular weight (MW) of proteins. However, 2-DE cannot efficiently separate some proteins such as the proteins with extreme isoelectric point (pI > 10 or pI <3) and the proteins with high molecular weight (M_W > 200 kDa). Liquid chromatography-tandem mass spectrometry (LC-MS/MS), another important analysis technology in proteomics research, can resolve such issues to some extent. 2-DE separates samples at protein level, while LC-MS/MS separates at peptide level. Thus, LC-MS/MS can circumvent some limitations of 2-DE, and is becoming a large-scale separation and identification technique. In the present study, we chose three methods commonly used in proteomics researches to extract whole proteins in cultured 293T cells, including Triton X-100 method, urea method and TRIzol method. The profiles of proteins obtained from LC-MS/MS were compared and further analyzed using bioinformatics analyses.

2 Experimental

2.1 Instrument and reagents

Allegra ™ X-22R Centrifuge was from Beckman Coulter, USA. CO_2 incubator was from Thermo Fisher, USA. Mini-cell vertical electrophoresis systems and GS800 image scanner were obtained from Bio-Rad, USA. High performance liquid chromatography (HPLC) system was from Eksigent Technologies, USA. TripleTOF 5600 mass spectrometer was from Applied Biosystems, USA

Protease inhibitor was purchased from Roche, Germany. Iodoacetamide, dithiothreitol (DTT), formic acid, ammonium bicarbonate (NH_4HCO_3), acetonitrile (HPLC grade), and Tris were purchased from Sigma Aldrich, USA. Nuclease was from GE Healthcare, USA. Sequencing grade trypsin was obtained from Promega, USA. Trizol Reagent was purchased from Invitrogen, USA. Bradford protein quantification reagent was purchased from Bio-Rad, USA. High glucose DMEM medium, Fetal bovine serum were from Hyclone, USA. Cell lysis buffer (# 9803) was purchased from Cell Signaling Technology, USA. Ultrapure water (18.2 MΩ cm, Milli Q) was used throughout the experiments. 293T cells were stored in liquid nitrogen in our laboratory. Other reagents were of at least analytical grade.

2.2 Experimental methods

2.2.1 Cell culture

293T cells were cultured in high glucose DMEM medium containing 10% fetal bovine serum at 37 °C (5% CO_2). Until the cell density reached 80%–90%, 293T cells were collected and dispensed into several centrifuge tubes equally at 2×10^6 cells per tube. The obtained cell solution was then centrifuged at 4 ºC for 10 min (500 × *g*). After removing the supernatant, the cell pellets were collected and lyzed using three different sample preparation methods, followed by protein extraction.

2.2.2 Extraction protocols of cell total proteins

2.2.2.1 Triton X-100 method

The cell lysis buffer (# 9803), with Triton X-100 as the main effective components, contains 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1%Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM *β*-glycerophosphate, 1 mM Na_3VO_4, and 1 μg mL^{-1} leupeptin. According to the instruction, cell pellet was lyzed in 100 μL of cell lysis buffer solution by pipetting repeatedly, and then centrifuged (12000 × *g*) for 10 min at 4 ºC. The supernatant was transferred to a new EP tube and kept at –80 °C.

2.2.2.2 Urea method

Lysis buffer solution containing 8 M urea (100 mM Tris-HCl, pH 8.0) was prepared. 100 μL of the buffer was used to lyze cell pellet with addition of 1 μL of nuclease and 10 μL protease inhibitor (10 ×). The supernatant was obtained by centrifugation (12000 × *g*, 10 min, 4 °C), then transferred into a new EP tube and kept at –80 °C.

2.2.2.3 TRIzol reagent method

According to the instruction, the Trizol reagent was added into cell pellet by pipetting repeatedly to lyze cells completely. After addition of chloroform, three layers could be obtained by centrifugation. After RNA in the upper aqueous phase was removed, DNA in the intermediate phase was precipitated by ethanol. Finally, proteins in the lower phase were precipitated