PROTEIN AND RNA EXTRACTION AND QUALITY ASSESSMENT FROM SMALL TISSUE SAMPLES USING TRIZOL

CRISTINA POP[1], SABINE AMELING[2], CRISTINA MOGOŞAN[1], FELICIA LOGHIN[3], ELKE HAMMER[2]

[1]Department of Pharmacology, Physiology and Pathophysiology, Faculty of Pharmacy, University of Medicine and Pharmacy Iuliu Haţieganu Cluj-Napoca, Romania
[2]Interfaculty Institute for Genetics and Functional Genomics, Ernst-Moritz-Arndt University of Greifswald, Germany
[3]Department of Toxicology, Faculty of Pharmacy, University of Medicine and Pharmacy Iuliu Haţieganu Cluj-Napoca, Romania

Abstract

Introduction. *In order to perform complex transcriptomic, genomic and proteomic analysis and gain deep insight into disease pathophysiology, relatively large amounts of sample are needed. Tissue available for study is often limited, especially human tissue. Concomitant extraction of RNA and proteins is important when differences in proteome and transcriptome have to be correlated. Trizol is a monophasic solution of phenol and guanidine isothiocyanate used for the extraction of nucleic acids but not yet popular for protein extraction. We have performed a comparative study in order to determine the reliability of Trizol RNA and protein extraction from small tissue samples.*

Methods. *Samples used were: mouse ventricle and human ventricle. A customized Trizol protocol was used to extract RNA and protein from the same sample. RNA quantitation (quantification, quantitation) and quality control (RNA integrity), protein quantitation and Mass Spectrometric (MS) analysis were performed. Interpretation of MS data was done with Proteome discoverer.*

Results and discussions. *Trizol extraction yielded sufficient RNA and protein quantity for further downstream applications. From both sources, high quality RNA and proteins was obtained, avoiding protein or RNA degradation. MS analysis revealed coverage of a wide span of sub-cellular protein components as expected and in concordance with other studies.*

Conclusions. *Trizol can be used successfully to extract RNA and protein from the same sample, limiting the amount needed. The results of the present study showed that Trizol can be used in specific cardiovascular disease studies in order to extract RNA and proteins from heart biopsies.*

Keywords: protein, RNA, extraction, small samples, Trizol.

Introduction

Genomic, transcriptomic and proteomic analysis of biological samples have become in the last years a common procedure to gain insight into the pathophysiology of disease. The extraction protocols usually are independent, time consuming and necessitate a relatively large amount of biological sample. In many cases, the amount of sample available is minuscule and has to be shared by investigators for several procedures, augmenting the need for effective procedures of extracting the interest molecules. The latest trend in omics technologies is the correlation of omics data, especially proteomic and transcriptomic data, in order to increase the reliability of the results generated by the software and to enable a holistic approach to the study of biological samples. Concomitant extraction of DNA, RNA and proteins from the same sample can provide the solution needed to solve both problems: diminish the amount of sample needed and allow the correlation of genomics data.

Adress for correspondence: alizarina_8popc@yahoo.com

Trizol is a reagent developed almost 25 years ago that uses phenol, guanidine thiocyanate and chloroform to isolate RNA in a single step method [1]. Using a customized Trizol protocol, we have isolated both RNA and proteins from the same biological sample. The aim of this study was to evaluate the quantity and quality of RNA and proteins extracted using the Trizol protocol in order to determine if we can use, in future studies, the two types of extracts for downstream applications.

Methods

Protein and RNA extraction

Biological samples used were mouse and human left and right ventricles. Mouse ventricle samples come from one healthy albino laboratory mouse and had one replicate for each ventricle. The amount or human ventricles available for analysis was so minute that replicates could not be used. The first step in the Trizol protocol was sample disruption and homogenization using liquid nitrogen. Biopsy was rapidly frozen in liquid nitrogen and then grinded into a fine powder. Subsequently, Trizol reagent was added and the material was again disrupted. The homogenates were incubated at room temperature for approximately 5 minutes, then chloroform was added and the vials centrifuged at 12.000g at 4ºC. Upper aqueous phase, containing RNA, was transferred to a new collection RNase-free tube. Both the remaining organic phase, containing protein and DNA, and the RNA phase can be stored overnight at 4ºC. In the remaining organic phase, DNA precipitation was performed using ethanol 100%. DNA pellet was discarded as it would not make the object of the present study. The supernatant, containing protein, was then transferred to a new collection tube. Further, proteins were precipitated using isopropanol and washed with guanidine hydrochloride in 95% ethanol. Proteins were re-suspended in UT solution (8M urea/2M thiourea). For RNA isolation, ethanol 100% was added to the aqueous phase. A Quiacube column (Quiagen, Valencia, CA) was used to bind RNA, followed by two washing steps and elution of RNA with RNase-free water.

Quantitation and quality evaluation

Total protein quantitation was performed using a Bradford assay kit (Pierce, Thermo Scientific, Bonn, Germany). Peptide mass spectrometry analysis was performed using the Orbitrap 4 (Thermo Scientific, Bonn, Germany), after protein reduction and alkylation, with DTT and iodoacetamide, respectively, and digestion with trypsin (1:10) as previously mentioned [2]. Proteins were identified via Proteome discoverer 1.3 (Thermo Scientific, Bonn, Germany) using a SEQUEST algorithm. Evaluation of RNA concentration and integrity was performed with by Nanodrop (Thermo Scientific, Bonn, Germany) and Bio-analyzer (Agilent Technologies, Santa Clara, United States).

Results

In this study we showed that protein and RNA extraction protocol from the same sample can result in enough quantity and high RNA and protein quality for downstream applications. It should be noted that the amount of sample used, both mouse and human, was a tip of a spatula, in order to mimic the situation of low sample amounts.

Table I shows the concentrations and total protein quantity extracted from each mouse heart sample. For mass spectrometry applications, we needed 4 µg protein, making the amount extracted sufficient. Figure 1 shows the total number of spectra, peptides and proteins identified in the MS analysis of the mouse heart samples. Among the two samples of left ventricle, no major differences could be observed, as similar protein quantities have been extracted. For the right V2 (right ventricle, second replicate), a greater sample quantity was used to begin with, resulting in a greater protein yield and more identifications, while for the right V1 (right ventricle, first replicate) protein extraction did not go well, resulting in the loss of proteins due to poor solubilization of the pellet. This result is consistent with other studies which describe difficulty in solubilization of centrifuged pellets though in these studies other solvents have been used such as sarkosyl in SDS (sodium dodecyl sulphate), urea in CHAPS (3-[(3-Cholamidopropyl) dimethylammonio]-1 propanesulfonate), trietanolamine-glycerol buffer with CHAPS [3].

Table I. Mouse ventricle Bradford assay results.

Sample	Protein Concentration	Protein Quantity
LeftV1	0.80 µg/µl	119.6 µg
RightV1	0.57 µg/µl	86.1 µg
LeftV2	0.79 µg/µl	110.9 µg
RightV2	1.64 µg/µl	246.0 µg

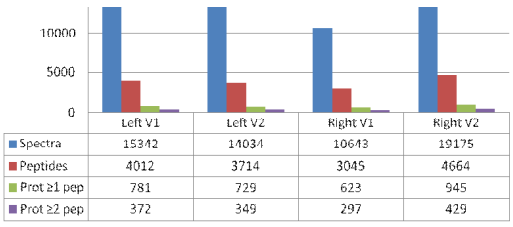

Figure 1 Mass spectrometry analysis results of mouse heart.

An analysis of the protein groups identified, using Proteome discoverer, revealed a great overlap between the samples: 577 protein groups overlapped in the left ventricles and 494 in the right ventricles. Non overlapping proteins represented less than 25% from the total amount of protein groups identified.

Table II. RNA evaluation for mouse heart.

Sample	RNA Concentration	RNA Quantity	RNA Integrity
LeftV1	44 ng/µl	1.76 µg	N/A
RightV1	34.7 ng/µl	1.39 µg	9.0
LeftV2	31.5 ng/µl	1.3 µg	7.9
RightV2	49.1 ng/µl	1.96 µg	8.0

Bio-analyzer results show an area under the peak of approximately 2:1 (18S:28S) for all mouse heart samples, a smooth baseline with no apparent small fragments and no degradation products. An RNA integrity score over 7.9 reveals sufficient RNA quality for downstream applications. The Nanodrop analysis revealed sufficient RNA quantity extracted (Table II). In correlation with the data resulted in the proteomics analysis, a larger amount of RNA could be observed in the right V2, due to a larger sample size to begin with.

Table III presents the concentrations and total protein and RNA quantity extracted from each human heart sample. There is a low protein and RNA yield, due to small sample size to begin with, especially in the left ventricle, but still there is sufficient protein and RNA amount for downstream applications.

Table III Bradford analysis results from human endomyocardial biopsy.

Sample	Protein concentration	Protein quantity	RNA concentration	RNA quantity
HEMB LiV	0.64 µg/µl	63.9 µg	15.5 ng/µl	620 ng
HEMB ReV	2.02 µg/µl	201.8 µg	38.4 ng/µl	1536 ng

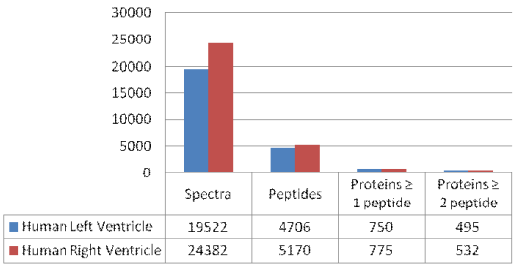

Figure 2. Mass spectrometry results human endomyocardial biopsy.

Mass spectrometry analysis reveals relatively similar protein identification between the two human heart samples (Figure 2) with an expected number of proteins identified. We experienced difficulty regarding RNA extraction due to DNA contamination. A DNA digestion step had to be integrated due to insufficient phase separation and more wash steps in order to eliminate contaminant and be able to further perform RT-PCR.

We feel confident that the proteins are intact after the extraction process, but in order to prove lack of protein degradation, future analysis must be performed, including gel electrophoresis and Western blotting.

Conclusion

In the present study we showed that concomitant RNA and protein extraction from heart biopsies is possible using a modified Trizol protocol. Our results are in concordance with other several reports and suport the use of this protocole as a robust method of extraction [4]. We have tried to reproduce the situation of low amount of sample and managed to obtain sufficient RNA and protein for downstream applications, of a relatively high quality. We showed that despite low sample amount, we could still process our samples and obtain relevant data.

This study represents a pilot study and its results will help us in future clinical cardiovascular studies with a holistic approach.

Acknowledgements

This study was carried out during a mobility programme financed by the University of Medicine and Pharmacy Iuliu Haţieganu Cluj-Napoca through the POSDRU 107/1.5/S/78702 scholarship.

Disclosures

Tissue samples used were from an approved study. Human subjects participating in the study had given the required informed consent. There is no conflict of interest.

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