Simultaneous isolation of total cellular lipids and RNA from cultured cells

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Lipid biochemists often need to analyze total cellular lipids as well as cellular messenger RNA (mRNA) levels. Conventionally, different cell samples are used for extraction of each class of biomolecule. Here, we describe a procedure for the simultaneous isolation of both total cellular lipids and total RNA from the same sample. The method is based on the fact that mild organic solvents efficiently extract lipids but do not disrupt/degrade cellular RNA. This procedure not only reduces the time and expense of analysis, but also allows a direct investigation of any correlation between lipid and transcript levels. While this method has only been applied in fibroblasts, prior delipidation of samples may be useful for extraction of nucleic acids from lipid-rich cells such as adipocytes. However, its application to other eukaryotic cell types needs to be tested. The method may not be useful in plant cells or bacterial cells, which are structurally quite different from eukaryotic cells.

Total lipids can be efficiently extracted from cultured cells using an organic solvent consisting of a 3:2 mixture of hexanes:isopropanol (1). While this method has been shown to completely extract various classes of lipids including fatty acids, steroids, phospholipids and triglycerides, it is not efficient for the extraction of gangliosides (2). Treatment of adherent cells with the solvent leaves behind a cell skeleton of nonlipid components from which RNA can be isolated using standard protocols. Cells that grow in suspension can be collected by centrifugation prior to extraction of lipids. This paper demonstrates the validity and feasibility of the technique for the simultaneous extraction of lipids and RNA from the same cell sample.

Normal human foreskin fibroblast (FSF) cells (GM04390) were obtained from the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository (NIGMS). They were maintained and plated as described (3). Confluent FSF cells from each well of a 12-well plate were extracted twice sequentially with 500 μL 3:2 hexanes:isopropanol for 13 min each on ice. The resulting lipid extract was evaporated under N₂ gas and resuspended in 100 μL dichloromethane (CH₂Cl₂). Analysis by thin layer chromatography revealed the presence of significant levels of cholesterol esters (CE) and phospholipids (PL) (see Figure 2A). The spot for free cholesterol (FC) was detected only when cells were preloaded with cholesterol. This protocol of lipid extraction is routinely used to quantitate the efflux of cellular cholesterol and phospholipids using cells loaded with radioactive lipids (4, 5).

The remaining delipidated cellular material, which remained adherent, was solubilized in TRI reagent (Sigma, St. Louis, MO, USA), and total cellular RNA was isolated according to TRI reagent-specific instructions (6). TRI reagent (400 μL) was added to each well of a 12-well plate, and the cellular material from 3 wells was combined for RNA isolation. The total yield of RNA from each delipidated sample was 5.78 μg.

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**Figure 1. Characterization of RNA isolated from delipidated cells.** (A) Assessment of RNA integrity by agarose gel electrophoresis. Different RNA preparations were resolved on a 2% agarose gel and stained with ethidium bromide (EtBr). Lane 1, RNA isolated from normal fibroblasts. Lane 2, RNA isolated from fibroblasts that had been delipidated by extraction with a 3:2 mixture of hexanes:isopropanol. Lane 3, RNA isolated from normal cells (as in lane 1) was subjected to treatment with 3:2 hexanes:isopropanol prior to resolution on the agarose gel. Lane 4, RNA (3 μg) isolated from delipidated cells (as in lane 2) was treated with RQ1 RNase-free Dnase (3 U in a volume of 100 μL) for 30 min at 37°C. The RNA was subjected to extraction with phenol:isoamyl alcohol:chloroform and was precipitated with sodium acetate and ethanol. (B) Densitometric analysis of lanes 1 and 2 of panel A. The relative intensity plots were obtained using the National Institutes of Health (NIH) Image software. The areas under the peaks indicate the relative abundance of each band. (C) Amplification of β-actin fragment by reverse transcription PCR (RT-PCR). RNA (1 μg) isolated from normal (lanes 3 and 4) or delipidated (lanes 1 and 2) cells was subjected to a reverse transcription reaction in the presence of the primer pair described in the text.
that delipidation of cells caused co-precipitation of some genomic DNA with the RNA. To investigate this possibility, the RNA sample prepared from delipidated cells was treated with DNase to digest any DNA present in the sample. Treatment with RQ1 DNase seemed to eliminate the high molecular mass band (Figure 1A, lane 4), indicating that RNA isolated from delipidated cells may be contaminated with DNA. However, a significant amount of RNA was lost during the reprecipitation step; thus, the disappearance of the high molecular weight band may be a nonspecific result of lower yield, independent of DNase treatment.

Absorbance values of RNA samples indicate that the total yield of RNA from normal cells is comparable to that from delipidated cells (Table 1). However, upon resolution by agarose gel electrophoresis, it appears that the RNA sample prepared from delipidated cells is contaminated with some high molecular weight components. In order to estimate the relative levels of each of the molecular species in the prep, the first two lanes in Figure 1A were subjected to a densitometric scan (Figure 1B). The top panel shows RNA isolated by the standard protocol, whereas the lower panel is a scan of RNA isolated from delipidated cells. Clearly, the intensities of the two prominent bands for 28S and 18S rRNA are similar in both lanes. An additional high molecular weight band (probably DNA or aggregated RNA) seen in the sample from delipidated cells is a relatively minor component and is absent from normally isolated RNA. While the presence of this contaminating species is a limitation of the proposed procedure, the scan shows that it does not contribute significantly to the overall yield of total RNA. More importantly, results described below suggest that the larger sized component does not compromise the integrity and utility of the RNA preparation.

Reverse transcription PCR (RT-PCR) was used as a representative assay to determine the utility of RNA isolated from delipidated cells for molecular biology applications. RNA from either normal or delipidated cells was used as a template to synthesize cDNA, using random hexamer primers and Moloney murine leukemia virus (MMLV) reverse transcriptase (Figure 1C, lanes 2 and 4). Amplification of contaminating DNA, if any, was assessed by reactions carried out in the absence of reverse transcriptase (Figure 1C, lanes 1 and 3). After reverse transcription, a 285 bp β-actin-specific fragment was amplified using sense primer 5′-TCATGAAGTGTGA CGTTGACATCCGT-3′ and antisense primer 5′-CTTAGAAGCATTTGC GGTTGACATCCGT-3′. There was no amplification in the absence of reverse transcriptase for either RNA sample.

Table 1. Comparison of RNA and Protein Obtained from Normal and Delipidated Fibroblasts

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<th>Normal</th>
<th>Delipidated</th>
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<tr>
<td>RNA yield (µg)</td>
<td>5.92 ± 1.1 (n = 38)</td>
<td>5.78 ± 1.5 (n = 34)</td>
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<tr>
<td>A₂₆₀/A₂₈₀</td>
<td>1.7 ± 0.12 (n = 38)</td>
<td>1.7 ± 0.19 (n = 34)</td>
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<tr>
<td>Protein yield (µg)</td>
<td>63 ± 3 (n = 3)</td>
<td>32 ± 2 (n = 2)</td>
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This result suggests that there is no significant contamination by functional DNA in the RNA prepared from delipidated cells. In the presence of reverse transcriptase, amplification of a β-actin fragment was equally efficient for both RNA samples, reaffirming the use of the proposed protocol for simultaneous isolation of lipids and RNA from the same cell sample.

Extraction of lipids from cells resulted in a significant loss of cellular proteins. The total protein yield from delipidated cells was only 50% of that obtained from normal cells (Table 1). This is expected since delipidation results in dissolution of the plasma membrane and loss of cytosolic proteins. Also, lipid-protein interactions in vivo may cause any proteins associated with lipids to be removed during delipidation. It is expected that delipidated cells may contain only cytoskeletal proteins. Cell lysates from normal and delipidated fibroblasts (3 μg each) were resolved by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and subjected to a sensitive silver-staining protocol (8) (Figure 2B). Lysates of normal cells contained many protein bands of molecular weight 35 kDa and higher. Lysates of delipidated cells showed detectable levels of only four proteins. These were the four most abundant protein bands seen in normal cells. Since equal protein mass, as determined by Lowry’s protein assay (9), was loaded for both lanes, it was surprising that the sample from delipidated cells showed a significantly less intense staining pattern than the sample from normal cells. Thus, some of the protein that was measured by the Lowry assay was either too large or too small to be detected by a 12% gel. The results indicate that delipidation either caused aggregation of proteins into large particles excluded by the 12% polyacrylamide gel or resulted in degradation of proteins into smaller fragments that ran off the 12% gel. These results clearly show that delipidated cells are not appropriate for analysis of cellular proteins.

This paper presents evidence that the conventional protocols for lipid extraction and RNA isolation can be sequentially applied to the same cell sample as a reliable method to analyze total cellular lipids and RNA from cultured cells. The RNA isolated from delipidated cells is equivalent to RNA isolated from normal cells and can be used in routine applications such as cDNA synthesis and RT-PCR as described here. In addition to the advantage of obtaining dual data from the same sample, the proposed protocol will result in significant time and cost savings for the lipid biochemist. Though not tested, it should be possible to use RNA isolated from delipidated cells for applications such as Northern blot analysis, RNase protection assays, microarray applications, and other molecular analyses. The protocol can likely also be used for isolation of total DNA. Furthermore, the delipidation protocol described here may be useful to circumvent the difficulty of isolating RNA from lipid-rich samples such as adipocytes (10). However, this method of RNA isolation has not yet been validated for all of these possible applications.

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COMPETING INTERESTS STATEMENT

The authors declare no competing interests.

REFERENCES