Silicon Carbide as a Novel RNA Affinity Medium with Improved Sensitivity and Size Diversity

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Abstract

Gene expression analysis using RNA has played a central role in our understanding of many aspects of the biology of an organism, including developmental timing, stem cell differentiation, signal transduction, disease and oncogenesis. In the past decade, studies of post-transcriptional gene regulation have brought attention to new classes of small RNA, including microRNA (miRNA) and small interfering RNA (siRNA). The importance of small RNAs is clearly demonstrated by their diverse functions and applications. In fact, the emergence of the field of RNA interference has led to a drastic change in the criteria for RNA sample preparation methodologies. It is now highly important to be able to isolate total RNA with all sizes of RNA, including miRNA. Interestingly, many commercially available total RNA extraction products, particularly those using silica as a separation medium, selectively exclude RNA smaller than 200 nucleotides. Additional steps, resulting in lengthy procedures, have to be incorporated to capture this important small RNA fraction. Here, we demonstrated that silicon carbide (SiC) can be utilized as a chromatographic medium for RNA purification. We showed that with the ues of SiC all sizes of RNA, including small RNA, can be isolated reproducibly with great sensitivity and ease. The true total RNA isolated using SiC, without the use of phase separation, consisted of similar quantity of miRNA to other miRNA isolation products utilizing a guanidium/phenol protocol. In addition, RNA isolation using SiC had a better linearity and sensitivity for both large messenger RNA (mRNA) and small miRNA when compared to a silica-based protocol or a traditional phase separation protocol. As SiC can be used in both a single column and high-throughput formats, it presents a robust solution to true total RNA isolation, particularly for rapid and simultaneous purification of miRNAs and their respective mRNA targets.

Introduction

In the past decade, the emergence of the field of RNA interference has led to a drastic change in the criteria for RNA sample preparation methodologies. All sizes of RNA, including microRNA, should be able to be isolated with ease from a sample such that the expression level of both small RNAs and their mRNA targets can be correlated. The traditional method for total RNA isolation is the acid phenol/ guanidine thiocyanate method (Chomczynski and Sacchi, 1987), commonly known today as the Trizol (Invitorgen, Carlsbad, CA) or TRI Reagent (Sigma-Aldrich, St. Louis, MO) method. This method delivers high yields of RNA and retrieves both small and large RNA species, however the method does rely on the use of harmful organic solvents and is rather time-consuming. During the last decade, there have been an increasing number of commercially available silica-based spin column products for total RNA isolation. The isolation of RNA using these kits is rapid and generally does not require the use of organic solvents. However, the sizes of the RNA isolated with these kits are larger than 200 nucleotides, as most of the small RNA species, including miRNA and siRNA, are selectively excluded. Additional steps, such as incorporation of an acid phenol extraction step, are required for the capturing of small RNA molecules onto the silica media. Thus an increasing demand exists for a novel RNA isolation technology that can combine the species diversity of Trizol/TRI Reagent and the ease of use of spin column chromatography.

Objectives of the Study

1. To demonstrate the use of silicon carbide (SiC) as an affinity medium for the purification of total RNA, including microRNAs, without use of phenol extraction.
2. To compare the performance of SiC in RNA purification to that of current technology including phenol-based extraction and silica-based extraction.

Results

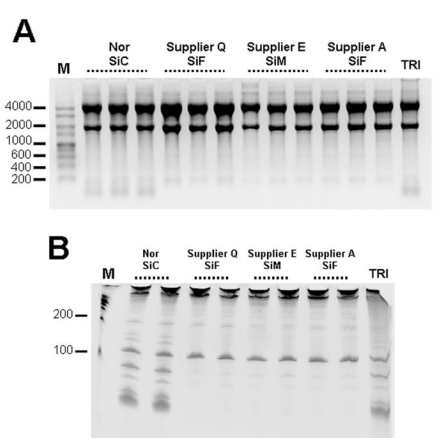

Figure 1. True Total RNA Isolation using SiC, without Phenol-Extraction. Total RNA was isolated from 1 x 10^6 HeLa cells using RNA isolation kits with spin column formats employing either silicon carbide (**SiC**), silica fiber (**SiF**) or silica matrix (**SiM**) as the separation medium. Guanidine thiocyanate/phenol-based TRI reagent isolation was used as positive control for isolating the complete size range of RNA. Equal portions of isolated RNA were resolved on (**A**) a 1.5% formaldehyde-agarose gel or (**B**) an 8% Urea-PAGE gel. All kits isolated similar amounts of RNA as suggested by the intensity of the major ribosomal RNA bands. Only total RNA isolated using SiC (Panel A Lanes 1-3, Panel B Lanes 1,2) or TRI reagent (**TRI**) (Panel A Lane 13, Panel B Lane 9) contained both the large and small RNA species, including those less than 200 nt. In contrast to SiC, the RNA isolated using silica (Panel A Lanes 4-12, Panel B Lanes 3-8) included very few RNA species less than 200 nt in size, even though all kits compared employed similar lysis conditions based on Chomczynski and Sacchi (1987). Nor = Norgen Total RNA Isolation Kit; TRI = Sigma-Aldrich TRI Reagent

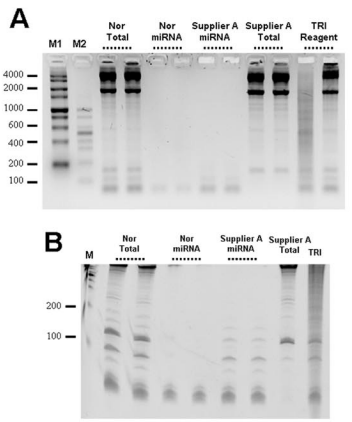

Figure 2. Total RNA Isolated using SiC Consists of a Similar Quantity and Diversity of Small RNA Species Compared to miRNA-Specific Products. Total RNA was isolated from 1 x 10^6 HeLa cells using Norgen's silicon carbide (**Nor Total**), or silica fiber (**Supplier A Total**) as the separation medium. Small RNA recovery was compared to two commercially available miRNA enrichment kits from the same suppliers (**Nor miRNA** and **Supplier A miRNA**). Guanidine thiocyanate/phenol-based TRI reagent isolation was used as a control for isolating the complete size range of RNA. Equal portions of isolated RNA were resolved on (**A**) a 1.5% formaldehyde-agarose gel or (**B**) an 8% Urea-PAGE gel. Only total RNA isolated using SiC (Panel A Lanes 1 and 2, Panel B Lanes 1 and 2) had the same amount of small RNA species as the kits that were specifically geared towards the purification and enrichment of microRNA (Panel A Lanes 3-6, Panel B Lanes 3-6) In contrast, total RNA isolated using silica fibers only contains RNA that is greater than 200 nt in size (Panel A Lanes 7 and 8, Panel B Lane 7). Enrichment of small RNA using SiC did not involve any phenol-extractions, which is in contrast to microRNA enrichment using silica fibers.

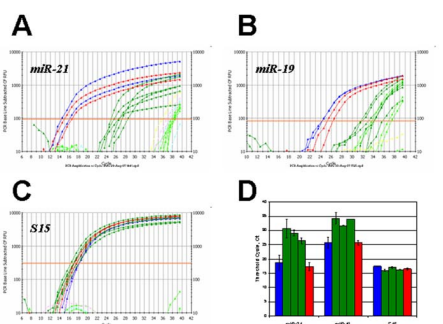

Figure 3. High Recovery of Specific microRNA in Total RNA Isolated using SiC. The relative abundance of specific microRNA in the total RNA isolated using various kits was compared. RNA isolated in the aforementioned comparison (Figure 1) was polyadenylated and used in RT-qPCR reactions using primers specific for the human *miR-21* (**Panel A**), and *miR-19* (**Panel B**). The resulting threshold cycle (C_T) values were summarized in **Panel D**. Total RNA isolated using SiC contained levels of microRNA that are comparable to the levels obtained when RNA is isolated using TRI Reagent, as reflected by the C_T values for the *miR-21* and *miR-19*. In contrast, the C_T values for the amplification of *miR-21* and *miR-19* using silica-purified total RNA are much higher, suggesting much less microRNA is present in these RNA samples. All the different separation materials amplified mRNA of *S15* at the same rate and gave similar C_T values (**Panel C**). ▲ = Silicon carbide-based Norgen Total RNA Kit; ▲ = Silica-based total RNA kits (Suppliers Q, E and A); ▲ = Sigma-Aldrich TRI Reagent. Curves in matching lighter colors represented corresponding –RT reactions and ▲ = No Template Control

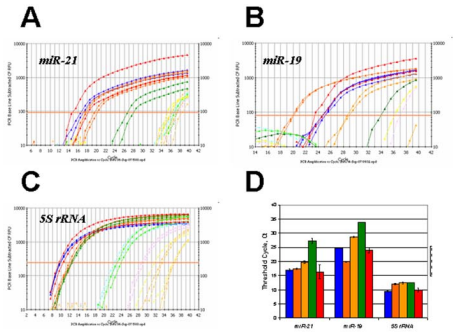

Figure 4. Recovery of Specific microRNA in Total RNA Isolated using SiC was Comparable to that Isolated using microRNA Enrichment Products. The relative abundance of specific microRNA in the total RNA isolated with SiC was compared to that from miRNA enrichment products. RNA isolated in the aforementioned comparison (Figure 2) was polyadenylated and used in RT-qPCR reactions using primers specific for the human *miR-21* (**Panel A**), and *miR-19* (**Panel B**). As indicated by the C_T values (**Panel D**), both the microRNA purification kits and the SiC-based total RNA kit recovered microRNAs (both *miR-19* and *miR-21*) that were very close to the levels obtained using TRI reagent. For the larger control 5S rRNA, all the different separation methods showed amplification with similar C_T values. Combining these results with the results in Figure 3, it can be seen that purification using SiC provides a new option for isolating a complete profile of RNA, including small microRNA. SiC allows for the simultaneous isolation and characterization of important microRNAs and their specific messenger RNA targets, without involving any phenol-based phase separation. ▲ = Silicon carbide-based Norgen Total RNA Kit; ▲ = Norgen microRNA Purification Kit; ▲ = Silica-based microRNA purification kit from Supplier A; ▲ = Total RNA purification kit from Supplier A; ▲ = Sigma-Aldrich TRI Reagent. Curves in matching lighter colors represented corresponding –RT reactions and ▲ = No Template Control

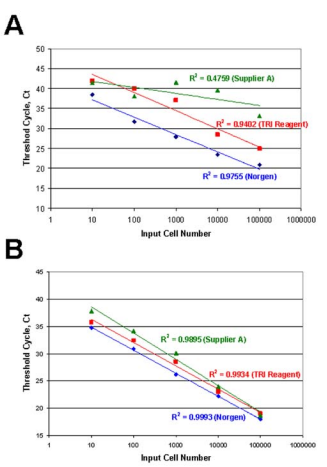

Figure 5. Total RNA Isolated using SiC Showed Better Linearity and Higher Sensitivity for both Large Messenger RNAs and Small microRNAs. Total RNA was isolated from increasing amounts of HeLa cells using a silicon carbide-based total RNA purification kit, a silica-fiber based total RNA purification kit, and guanidine thiocyanate/phenol-based TRI reagent as a control. The purified total RNA were then used in RT-qPCR to detect the microRNA *miR-21* (Panel A) and the mRNA *S15* (Panel B). Silicon carbide columns were able to isolate both higher and more consistent levels of *miR-21* transcripts over a wide input range of cells, down to as little as 10 cells (R^2 values: SiC = 0.9755 >> TRI = 0.9402 >> SiF = 0.4759). All three total RNA samples gave liner results for large messenger RNA species (human *S15* transcript tested), indicating the consistency of RNA isolation for all the three methods. Importantly, SiC-isolated total RNA again showed both the lowest C_T values, as well as the highest R^2 value. ▲ = Silicon carbide-based Norgen Total RNA Kit; ▲ = Silica-based commercial total RNA kits (Supplier A); ▲ = Sigma-Aldrich TRI Reagent.

Conclusions

1. SiC-based RNA purification effectively isolated a complete profile of RNA species, from large messenger RNA to small microRNAs, without the use of phenol-extraction.

2 SiC-based RNA purification isolated both mRNA and miRNA with higher sensitivity as well as better linearity when compared to existing RNA purification technologies.

References

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