Published in final edited form as:

Anal Biochem. 2007 June 1; 365(1): 147–148.

Application of RNase in the purification of RNA-binding proteins

Jonghoon Kang¹, Myung Soog Lee, and David G. Gorenstein*

Sealy Center for Structural Biology and Department of Biochemistry and Molecular Biology, The University of Texas Medical Branch, Galveston, Texas 77555-1157, USA

Abstract

Basic findings—It was found that RNA-binding proteins can be contaminated with host RNA during purification. The contamination of purified RNA-binding protein with RNA was identified by gel electrophoresis and EtBr staining. Our data suggest that applications of appropriate enzymes (DNase or RNase) in the early stage of purification may remove the contaminating nucleic acids.

Significance—The concept introduced in this research can easily be extended to the purification of other RNA- or DNA-binding proteins by applying RNase or DNase directly to the cell extracts.

Keywords

Protein purification; RNA-binding protein; hnRNP C1; RNase

Purification of an (over)expressed protein is an essential requirement for the subsequent biochemical and biophysical characterization of the protein. For this purpose, purity and maintaining native conformations of the protein are the most important criteria. Especially if the protein can bind to other molecules in an expression system, these bound molecules, which are considered as contaminants, should be removed along the purification. The contamination problem will be more prominent when the expression level of protein is higher so that it acquires an increased chemical potential for binding [1] or the binding specificity is broader. For example, DNA-binding proteins can be contaminated with DNA in a host, which is usually *E. coli*, and it can distort the downstream studies of the protein. King *et al.* [2], for instance, utilized the ability of high salt (1 M NaCl) to disrupt interactions between NF- κ B and short pieces of bacterial DNA created by sonication. Using this method, they found that no contaminants DNA remain in the purified NF- κ B.

In this study, we report RNA contamination of heterogeneous nuclear ribonucleoprotein C1 (hnRNP C1) protein purified with a commercial purification system and propose a way to resolve the contamination problem. As a member of hnRNPs, hnRNP C1 is involved in pre-mRNA packaging, spliceosome assembly and in nuclear retention of unspliced hnRNA [3]. As the protein is known to have broad specificity in binding to RNA [4], it may be bound to host RNA or DNA during overexpression. In our expression and purification system, we identified that the purified protein was contaminated with RNA and we were able to remove the contaminating RNA by applying a mixture of RNases directly to the cell extracts.

*Corresponding author, David G. Gorenstein, Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, Texas USA 77555-1157, Telephone: 409-747-6800, Fax: 409-747-6850, E-mail: dggorens@utmb.edu

¹Current address, Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA. USA 24061 (jkang@vbi.vt.edu)

This submission is original and is not under consideration elsewhere.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The cDNA of hnRNP C1 was subcloned into the *Nde I* and *Sap I* site of the pTYB1 vector of IMPACT-CN system (New England Biolabs, Beverly, MA), and the resulting plasmid was transformed into BL21(DE3) pLysS cells. The cells were grown in Luria-Bertani broth (2 l) with 50 µg/ml ampicillin, 34 µg/ml chloramphenicol at 37 °C. When the OD₆₀₀ of the culture reached 0.8 ~ 1.0, IPTG2 was added to a final concentration of 1 mM to induce protein expression. The culture was transferred to a 25 °C air shaker for 18 ~ 20 h.

After culture, the protein was purified following the manufacturer's protocol. The cells were harvested and resuspended in 100 ml of lysis buffer (20 mM Tris (pH 7.5), 2 M NaCl, 1 mM EDTA2, 0.1% Triton X-100, 0.1% Tween 20) with a complete protease inhibitor cocktail (Cat. # 1 697 498, Roche, Indianapolis, IN). The cell suspension was sonicated on ice for 30 min. The supernatant (soluble fraction) was separated from the cell debris by centrifugation at 14000x g for 40 min at 4 °C, and the supernatant was filtered through a 0.2-micron filter. The clarified cell lysate (~ 70 ml) was slowly loaded at 0.5 ml/min onto a chitin column (~ 20 ml) equilibrated with column buffer (20 mM Tris (pH 7.5), 2 M NaCl, 1 mM EDTA). After the supernatant was loaded onto the column, the flow rate was raised to 2 ml/min and the column was washed with 10 bed volumes of column buffer. Then, the column was washed with 3 bed volumes of cleavage buffer (20 mM Tris (pH 8.8), 0.5 M NaCl, 1 mM EDTA). Induction of the on-column cleavage was conducted by quickly flushing the column with 3 bed volumes of the cleavage buffer (20 mM Tris (pH 8.8), 0.5 M NaCl, 1 mM EDTA) containing 50 mM of DTT2. The column was sealed and intein autoproteolysis was allowed to proceed at 37 °C for 24 h. After cleavage, the released hnRNP C1 was eluted with 3 bed volumes of cleavage buffer and pooled. The collected protein was then dialysed against PBS2 (pH 7.4).

After dialysis, the protein sample was treated with either DNase I (Cat. # M0303S, New England Biolabs, Beverly, MA) or RNase A (Cat. # 19101, Qiagen, Valencia, CA) and the reaction mixture was combined with 6x gel-loading buffer (0.25% bromophenol blue and 30% glycerol) and electrophoresed on an 10% polyacrylamide gel in 1x tris-borate/EDTA buffer at 200 V for 20 min. After EtBr2 staining, the gel image (Fig. 1A) was taken by a CCD2 camera [5]. As Fig. 1A shows, the protein sample purified even with high-salt buffers (2 M NaCl) was contaminated with nucleic acids, which were resistant to a DNase treatment. However, the nucleic acid was not detected in an RNase-treated sample indicating the contaminant was RNA. This proves that RNA-binding proteins can be contaminated with host RNA during purification.

To resolve the problem a mixture of RNases (Cat. # 2288 Ambion, Austin, TX) was applied in the early stage of purification right after centrifugation following sonication. Specifically 500 µl of the RNase mixture was added to 100 ml cell lysate and the lysate was incubated overnight at 4 °C and the protein was purified as above but using buffers containing 0.5 M NaCl as recommended by the manufacturer instead of 2 M NaCl. The purified protein was examined and no contaminating RNA was detected (Fig 1B). To our knowledge, this appears to be new from the literature.

In summary, the contamination of purified RNA-binding protein with RNA was identified by gel electrophoresis and EtBr staining. The concept introduced in this research can easily be extended to the purification of other RNA- or DNA-binding proteins by applying RNase or DNase directly to the cell extracts. Based on our results, we recommend that purified nucleic acid-binding proteins need an investigation of the potential contaminations with host nucleic acids. If there is contamination detected, applications of appropriate enzymes (DNase or RNase) in the early stage of purification may remove the contaminating nucleic acids. Finally, although the added nucleases are expected to be removed during purification, one has to check the purified protein sample for any remnant nuclease activity.

Acknowledgements

This work was supported by grants from the U.S. Edgewood Chemical Biological Center (W911 SR-04-C-0065), NIH (AI27744 and U01 AI054827), NHLBI (N01HV28184), and the Welch Foundation (H1296). The cDNA of hnRNP C1 was generously provided by Dr. Arthur M. Krieg (Coley Pharmaceutical Group).

References

1. Chang, R. Physical chemistry for the chemical and biological sciences. University Science Books; Sausalito, CA: 2000.
2. King DJ, Bassett SE, Li X, Fennewald SA, Herzog NK, Luxon BA, Shope R, Gorenstein DG. Combinatorial Selection and Binding of Phosphorothioate Aptamers Targeting Human NF-B RelA (p65) and p50. *Biochemistry* 2002;41:9696 – 9706. [PubMed: 12135392]
3. Dreyfuss G, Matunis MJ, Pinol-Roma S, Burd CG. hnPNP proteins and the biogenesis of mRNA. *Annu Rev Biochem* 1993;62:289–321. [PubMed: 8352591]
4. Brunner JE, Nguyen JH, Roehl HH, Ho TV, Swiderek KM, Semler BL. Functional interaction of heterogeneous nuclear ribonucleoprotein C with poliovirus RNA synthesis initiation complexes. *J Virol* 2005;79:3254–3266. [PubMed: 15731220]
5. Kang J. Application of a cooled charge-coupled device (CCD) camera for the detection of chemiluminescence signal. *Biologicals*. in press

Abbreviations used

CCD	charge-coupled device
DTT	dithiothreitol
EDTA	ethylene diamine tetraacetic acid
EtBr	ethidium bromide
IPTG	isopropyl β -D-1-thiogalactopyranoside
PBS	phosphate-buffered saline

Fig 1.

(A) Contamination of purified hnRNP C1 protein with RNA. Lane 1 is 5 μ l of DNA size marker (Cat. # 170-8200, BIO-RAD, Hercules, CA). 20 μ l of purified hnRNP C1 protein (560 nM) was loaded onto the gel without enzyme treatments (lane 2), after a treatment with DNase I for 37 $^{\circ}$ C for 2 h (lane 3) or RNase A for 25 $^{\circ}$ C for 2 h (lane 4), respectively, before loading onto the gel. RNA was detected in lanes 2 and 3. (B) Lane 1 is 5 μ l of the DNA size marker. Lane 2 is 20 μ l of purified hnRNP C1 protein (1.8 μ M). No RNA was detected in lane 2. The gel images were inverted.