Nucleic Acids Research, 1996, Vol. 24, No. 18 **3647–3648**

Preparative-scale purification of RNA using an efficient method which combines gel electrophoresis and column chromatography

Lynette Cunningham, Kirk Kittikamron and Yi Lu*

Department of Chemistry, University of Illinois at Urbana–Champaign, 505 S. Mathews Avenue, Urbana, IL 61801, USA

Received June 27, 1996; Accepted July 31, 1996

ABSTRACT

Here we describe a reliable method for purifying large amounts of RNA of any sequence and length with comparable efficiency and resolution of gel electrophoresis and with capacity approaching that of column chromatography. The RNA mixture of interest is separated on a cylindrical denaturing polyacrylamide gel, eluted by a peristaltic pump, detected by a UV-vis detector, and collected by a fraction collector. Using this method, we were able to separate one third of a 100 ml *in vitro* transcribed 34mer hammerhead ribozyme (~6.2 mg) in a single run. The entire 100 ml transcribed RNA (~18.5 mg) was separated after consecutive runs using one single gel preparation.

The increasing interest in the structure and function of RNA has created a demand for a reliable, preparative-scale purification method. For example, X-ray crystallography and spectroscopic studies require milligram quantities of pure RNA in order to provide useful structural information. Large amounts of pure phosphorothioate RNA are also needed for clinical trials. The two most widely used methods for large-scale purification are column chromatography (1,2) and denaturing polyacrylamide gel electrophoresis (PAGE) (3,4). While column chromatography offers high capacity and good resolution in purifying short oligoribonucleotides, denaturing PAGE is typically the method of choice in purifying longer (>30mer), *in vitro* transcribed RNA. However, the loading capacity of the conventional slab PAGE gel is small. For example, protocols used for obtaining 5–7 mg of pure ribozyme for NMR characterization require application of the crude transcript to eight (40 × 60 × 0.3 cm) polyacrylamide gels (4). Despite this limitation, no significant progress has been made in improving the efficiency of this technique for preparative-scale purification.

Here we describe a method that combines the best features of both gel electrophoresis and column chromatography. The method centers on the adaptation of the Model 491 Prep Cell (BioRad, CA), originally designed for protein purification. The RNA mixture of interest is separated on a cylindrical denaturing polyacrylamide gel, eluted by a peristaltic pump, detected by a UV-Vis detector, and collected by a fraction collector (Fig. 1D). Using this method, approximately one third of a 100 ml scale *in vitro* RNA transcription product (~6.2 mg) was separated in a single run. The entire 100 ml transcribed RNA (~18.5 mg) was separated after consecutive runs using one single gel preparation.

Figure 1A–C shows the effectiveness of this method in purifying a 34mer *trans*-acting hammerhead ribozyme that was prepared by *in vitro* transcription using a synthetic DNA template and T7 RNA polymerase (5). The crude 100 ml transcription mixture was ethanol-precipitated and redissolved in 2× TBE buffer (178 mM Tris-borate, 4 mM EDTA). After concentration with a Centricon-3 unit (Amicon, MA), a portion of the RNA was applied to a 20% denaturing polyacrylamide cylindrical gel prepared in the Prep Cell apparatus. The chromatogram of a typical Prep Cell run (Fig. 1A) displays two major peak groups, one eluting at 200 min and the other at 800 min. Three runs of increasing scale (0.46, 1.4 and 6.2 mg) were carried out. The overlay of the chromatograms from these runs (Fig. 1B) shows that the second peak group was resolved into a doublet. Four fractions of the second peak from each run (Fig. 1B) were applied onto an analytical 20% polyacrylamide slab gel. The gel patterns shown in Figure 1C are consistent with the conclusion that the first peak of the doublet (fraction 2 of each run) is the pure full-length RNA transcript (*n*). The fractions eluted before the peak (fraction 1 of each run) contain *n*–1 and *n*–2 transcripts while the fractions after the peak (fractions 3 and 4) contain *n* and *n* + 1 transcripts, respectively.

Overall, this method combines the best features of both gel electrophoresis and column chromatography. On one hand, provided that the appropriate percentage of acrylamide is chosen, this method could replace the commonly used PAGE slab gel method to purify RNA of any sequence and length with comparable efficiency and resolution. On the other hand, the capacity of this method approaches that of column chromatography. Compared with the 'crush-and-soak' and the electroelution methods of slab gel electrophoresis, the Prep Cell method does not require destruction of the gel. Consequently, the gel can be used several times for successive RNA purification, further reducing both time and expense. Our preliminary results have shown that the gel could be reused for up to three consecutive runs with no observable loss of resolution and yield. The handling procedures for the Prep Cell apparatus are straightforward, and the automatic fraction collector makes it possible for unattended operation. The purity of the final product can be controlled by

* To whom correspondence should be addressed

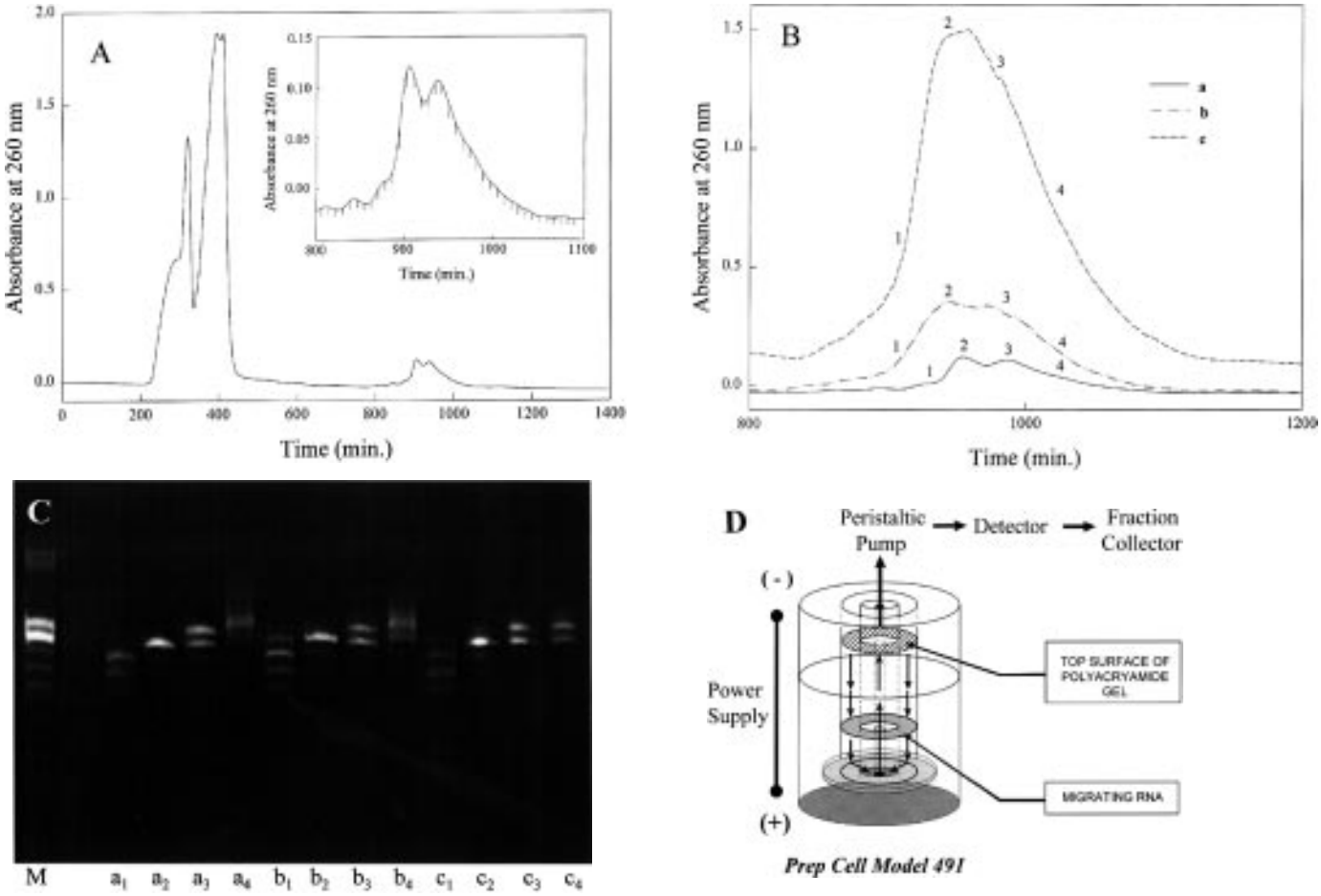

Figure 1. Results from purification of a 34mer *trans*-acting hammerhead ribozyme (5′-GGCGACCCUGAUGAGGCCGAAAGGCCGAAACAUU) on the Prep Cell. (**A)** Prep Cell chromatogram from run 'a'. The RNA was prepared in a 100 ml transcription mixture consisting of 200 nM DNA template, 4 mM NTP, 1 mg T7 RNA polymerase, 25 mM $Mg(NO_3)_2$, 5 mM DTT, 1 mM spermidine, 0.01% Triton X-100, 40 mM HEPES, pH 8.0. After incubation at 37°C for 6 h, the transcription was terminated by addition of 0.5 M EDTA. The RNA was isolated by ethanol precipitation and dissolved in 2× TBE. One twenty-fourth (~0.46 mg) of the crude mixture was concentrated in a Centricon-3 (to remove excess NTPs and thus improve RNA solubility) and loaded onto a 20% polyacrylamide gel in the Prep Cell. Separation conditions: 12 W constant power, 1 ml/min flow rate, 8 ml/fraction, 3 OD detector limit, 1.5× TBE electrophoresis buffer. Inset: expansion of the transcript peak along with fraction designations (8 ml each). **(B)** Overlaid Prep Cell chromatograms from runs 'a' (1/24), 'b' (1/12) and 'c' (1/3 of the crude transcript), corresponding to 0.46, 1.4 and 6.2 mg, respectively. The numbers 1, 2, 3 and 4 indicate respective fractions chosen for the analytical slab gel analysis shown in (C). Due to the difference in the amount of sample loaded onto the gel and the exact gel length, the retention time of each run can vary. Consequently, the chromatogram of run 'a' has been adjusted by +40 min for the purpose of comparison. **(C)** Analysis of Prep Cell peak fractions. Fractions (0.5 µg in each lane) were loaded onto an analytical 20% polyacrylamide slab gel containing 8 M urea (19 cm × 29 cm × 0.5 mm) and stained with ethidium bromide. Lane M: crude transcript; lanes a_1–a_4, b_1–b_4 and c_1–c_4: four representative fractions from runs 'a', 'b' and 'c', respectively. **(D)** Schematic diagram of the BioRad Prep Cell Model 491 design (gel tube: 3.7 cm internal diameter; 8.2 cm^2 gel surface area; 13 cm height).

varying the fraction size and by carefully selecting fractions of desired purity. The resolution and capacity could be further improved by building a system with a longer gel length. Given the efficiency and reproducibility of this method, it should greatly facilitate rapid purification of RNA in milligram quantities.

ACKNOWLEDGEMENTS

We thank J. J. Dunn and A. H. Rosenberg for providing the plasmid pAR1219 containing the T7 RNA polymerase gene, Gretchen Peterson for purification of the T7 RNA polymerase, Pascale Legault and Arthur Pardi for providing the RNA transcription protocol, Daniel Celander, Olke Uhlenbeck and Harry Noller for helpful suggestions, and Richard Gumport for use of gel digitalization equipment and critical reading of the manuscript. This research was supported by the NIH FIRST Award (GM53706), the Beckman Young Investigator Award, and the Donors of the Petroleum Research Fund, administered by the American Chemical Society.

REFERENCES

1 Anderson, A. C., Scaringe, S. A., Earp, B. E. and Frederick, C. A. (1996) *RNA*, A Publication of the RNA Society **2**, 110–117.
2 Wincott, F., Direnzo, A., Shaffer, C., Grimm, S., Tracz, D., Workman, C., Sweedler, D., Gonzalez, C., Scaringe, S. and Usman, N. (1995) *Nucleic Acids Res.* **23**, 2677–2684.
3 Pley, H. W., Lindes, D. S., DeLuca-Flaherty, C. and McKay, D. B. (1993) *J. Biol. Chem.* **268**, 19656–19658.
4 Heus, H. A., Uhlenbeck, O. C. and Pardi, A. (1990) *Nucleic Acids Res.* **18**, 1103–1108.
5 Milligan, J. F., Groebe, D. R., Witherell, G. W. and Uhlenbeck, O. C. (1987) *Nucleic Acids Res.* **15**, 8783–8798.