Vol. 144, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1987 Pages 271-276

CARRIER RNA ENHANCEMENT OF RECOVERY OF DNA FROM DILUTE SOLUTIONS

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Received February 26, 1987

SUMMARY. A study of the use of carrier RNA to improve precipitation of DNA from dilute solutions was conducted to define the conditions which optimize DNA recovery. Replicate samples containing labeled pBR322 and increasing concentrations of commercially-available Torula yeast RNA were ethanol precipitated at -20°C for 1 h in microfuge tubes obtained from various manufacturers. Nucleic acids were pelleted by centrifugation for either 5 or 30 min, dried and resuspended. Although recovery was not identical in each type of microfuge tube, in all cases the percent recovery increased when carrier was added. In most cases, extending centrifugation to 30 min did not significantly increase recovery.

Recovery of unlabeled DNA's of heterogeneous molecular weight and conformation was also enhanced by the addition of carrier RNA. DNA's recovered by this method can be successfully digested with BamHI and ligated with T4 DNA ligase.

The precipitation of nucleic acids with ethanol in the presence of sodium or ammonium ions is a commonly used technique which can be employed to concentrate dilute solutions of nucleic acid. This method can also be used to remove impurities such as detergents, heavy metals, or other components of the buffer which may interfere with subsequent treatment of the nucleic acid.

Procedures for precipitation of DNA should (i) provide a means for maximizing recovery of DNA, (ii) be rapid and simple, and (iii) allow subsequent manipulation of the DNA. Recently, investigators have evaluated existing procedures in an effort to design a protocol suitable for precipitating DNA from very dilute solutions (1,2). The primary problem associated with precipitation of DNA from low-concentration solutions is the poor recovery of DNA, possibly due to the small size of the precipitated DNA-aggregate.

The use of tRNA to enhance recovery of DNA by increasing the effective concentration of nucleic acids has been reported previously (3,4). The primary advantage of RNA carriers is the ease of their removal subsequent to ethanol precipitation. In addition, RNA carriers and the enzymes used to digest them are

0006-291X/87 $1.50

heat stable and therefore can be safely boiled to inactivate contaminating deoxyribonucleases.

Despite the widespread use of RNA carriers in the precipitation of DNA, there are no reports which describe the conditions for optimal utilization of this method. Therefore we undertook a study of the optimization of recovery of DNA from dilute solutions using Torula yeast RNA as a carrier. RNA-DNA mixtures were precipitated from solution by the addition of ammonium acetate and ethanol followed by incubation at -20°C. Resulting insoluble nucleic acids were sedimented by centrifugation. The pellet was then dried and resuspended.

In the work described here, we report the effects of carrier concentration, length of centrifugation, and type of microfuge tubes on DNA recovery. The utility of this method for precipitation of DNA's of heterogeneous size and conformation are also discussed.

MATERIALS AND METHODS

Nonradioactive carrier RNA Stock solutions of Torula yeast RNA (Sigma, Type VI) were prepared by dissolving RNA in water and titrating to pH 11.0 with 1 N NaOH. After dissolution of the RNA, the solution was titrated to pH 5.0 with HCl, autoclaved to inactivate nucleases, and stored at -20°C. The concentration of nucleic acids was determined by measuring the absorbance at 260 nm. Absorption by contaminating protein was determined and the value for nucleic acid concentration was adjusted using the method described by (5).

Determination of recovery of RNA-coprecipitated, labeled plasmid DNA Using a nick translation kit obtained from New England Nuclear, supercoiled pBR322 was radiolabeled with [methyl-1', 2'-^{3}H] dTTP according to the manufacturer's instruction and purified from unincorporated nucleotides by gel filtration chromatography through Sephadex G-100 (Sigma). Five µl aliquots of carrier RNA were added to 45 µl of100 ng/ml labeled pBR322 (600 cpm/ng) such that the final concentration of carrier RNA in these solutions were 0, 50, 500, and 1000 µg/ml. Two replicates of these four samples were prepared in microfuge tubes obtained from the following manufacturers: Brinkmann Instruments, PGC Scientifics, Treff Lab, VWR Scientific, and West Coast Scientific. One half volume of 7.5 M ammonium acetate was added followed by the addition of 2.5 volumes of cold 95% ethanol. The tubes were vortexed briefly and incubated at -20°C for 1 h. The nucleic acids were pelleted at 15,000 X *g* and 4°C in an Eppendorf Model 5415 centrifuge. One set was centrifuged for 5 min and the other for 30 min.

Following centrifugation, supernatants were removed and counted in a Beckman LS6800 liquid scintillation counter. The microfuge tubes were dried under vacuum and the pellets were dissolved in 260 µl of buffer (10 mM Tris, pH 7.5, 1 mM EDTA). To insure that resuspension was complete, the tubes were incubated at 37°C for 2 h. The solution was then removed and counted. Percent recovery was defined as the ratio of the amount of radioactivity in the pellet to the amount of radioactivity of a nonprecipitated sample.

After the resuspended pellet was removed, an equal volume of 0.25 N HCl was added to the empty tube, and the acid containing tube was incubated in a boiling water bath for 15 min. The solution was then neutralized by the addition of an equal volume of 1 M Tris, pH7.5, 1.5 M NaCl and counted in a liquid scintillation counter.

Digestion and ligation of plasmid DNA coprecipitated RNA An aliquot of 100 µl of unlabeled, supercoiled pBR322 (200 ng/ml) was ethanol precipitated in the presence of 500 µg/ml RNA, and the solution was concentrated tenfold as described above. RNase T1 (2 units/ml) and RNase A (4 units/ml) were added to the resuspended pellet and the solution was incubated at 37°C for 1 h. The plasmid was digested with BamHI then recircularized with T4 DNA ligase according to manufacturer's instructions. The products of these reactions were analyzed by agarose gel electrophoresis.

Determination of recovery of RNA-coprecipitated, unlabeled DNA Aliquots containing 100 µl of 1µg/ml of either Escherichia coli V517 plasmid DNA or HindIII digested λ-phage DNA, each containing either 0 or 500 µg/ml RNA, were precipitated as described above. The samples were centrifuged for 5 min, and the supernatants were discarded. After drying under vacuum, the pellets were resuspended in one tenth the original volume of buffer. The recovery was determined by comparing the intensity of fluorescence of aliquots electrophoresed on agarose gels.

RESULTS AND DISCUSSION

Effect of carrier RNA on recovery of supercoiled, open circular, and linear DNA. Dilute solutions of supercoiled and open circular DNA's isolated from E. coli V517, a strain that contains eight plasmid species ranging in size from 1.36 X 10^6 to 35 X 10^6 (6); and HindIII digested of λ-phage DNA, which generates fragments that range in size from 0.08 X 10^6 to 15 X 10^6, were precipitated in the presence of 1000 µg/ml RNA. After centrifugation, the pellets were resuspended in one-tenth the original volume and examined by agarose gel electrophoresis. Typical results are shown in Figure 1. Lanes a through c contained V517 plasmid DNA's. For comparison, a nonprecipitated sample is shown in lane a. Samples precipitated with and without carrier RNA are shown in lanes b and c respectively. By comparing the intensity of bands in lanes b and c, it can be clearly seen that the recovery of each plasmid species is enhanced when carrier RNA is added. In addition, the migration rate of plasmids coprecipitated with RNA appears to be unchanged relative to unprecipitated plasmid, indicating that the conformation of the plasmids has not been altered by this manipulation. HindIII restricted λ-phage DNA is shown in lanes d through f. An unprecipitated sample is shown in lane d. Lanes e and f contain samples precipitated with and without RNA respectively. Here again, the improved recovery of DNA's coprecipitated with RNA is clearly evident and is not restricted to a specific size class. Because of poor recovery, bands in lane f are not detectable in this photograph.

Digestion and ligation of RNA-coprecipitated plasmid DNA. Subsequent to RNA coprecipitation, a solution of resuspended, unlabeled pBR322 and carrier RNA was treated with RNase A and RNase T1. The plasmid DNA was then restricted with BamHI and analyzed by agarose gel electrophoresis. The digestion was found to be complete (data not shown). The linearized plasmid was then recircularized with T4

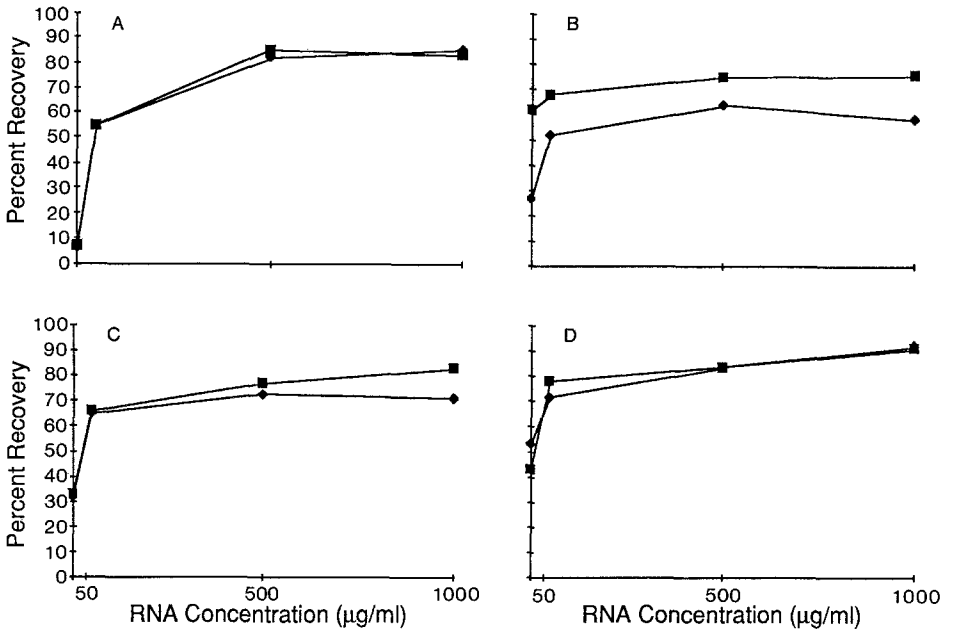

Fig. 1. Analysis of recovery of ethanol precipitated DNA's. Lanes a-c contain plasmid DNA's isolated from E. coli V517 which range in size from 1.36 X 10^6 to 35 X 10^6. Lanes d-f contain restriction fragments generated by HindIII digestion of λ– phage. Fragments range in size from 0.08 X 10^6 to 15 X 10^6. Aliquots of 100 μl of 1 μg DNA/ml containing 0 or 500 μg carrier RNA/ml were precipitated and resuspended in one-tenth the original volume; 4 μl aliquots were appled to a 0.8 % agarose gel slab and electrophoresed at 50 V. For comparison, unprecipitated aliquots containing 40 ng of DNA are shown. Lane a, unprecipitated E. coli V517 plasmid DNA's; lane b, precipitated with 500 μg RNA/ml; lane c, preciptated without carrier RNA; lane d, unprecipitated HindIII restricted λ–phage DNA; lane e, precipitated with 500 μg/ml RNA carrier; lane f, precipitated without carrier RNA.

DNA ligase and examined by agarose gel electrophoresis. Only the open circular form of pBR322 could be detected in the ligated sample.

Effects of carrier RNA on recovery of nick-translated plasmid DNA. Using the technique of radiolabeling, we were able to quantitate recovery of DNA coprecipitated with increasing concentrations of RNA and to assess the effect of extended centrifugation on recovery. Representative results are shown in Figure 2 A-D. Microfuge tubes were obtained from several manufacturers; and in one case, tubes from different lot numbers were used. Although recovery was not identical in each type of microfuge tube, in all cases the percent recovery increased when carrier RNA was added. The percent recovery was as follows: 6.4-62% at 0 μg/ml RNA, 46-78% at 50 μg/ml RNA, 64-85% at 500 μg/ml RNA, and 58-93% at 1000 μg/ml RNA. Results obtained using microfuge tubes from Brinkmann Instruments (data not shown) were similar to results shown in Figure 2A; when microfuge tubes from PGC Scientifics (data not shown) were used, we obtained results similar to those shown in

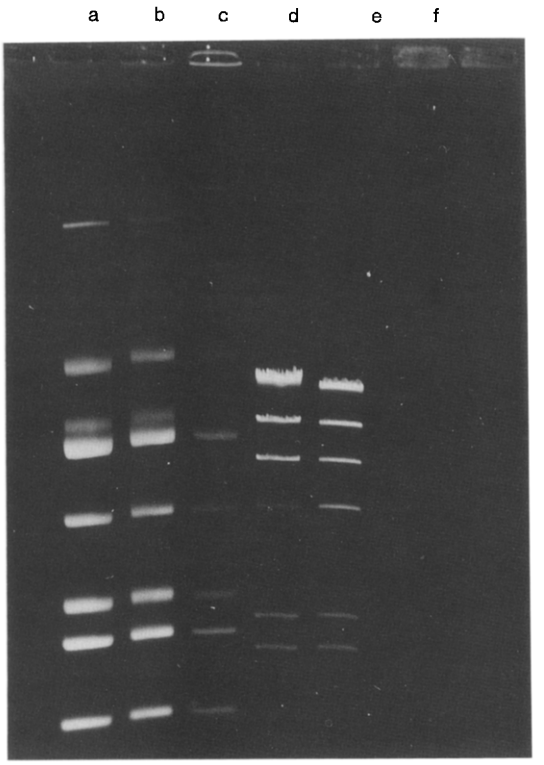

Fig. 2. Effect of RNA concentration and length of centrifugation on recovery of ethanol-precipitated, nick translated pBR322. Percent recovery is defined as the ratio of the amount of radioactivity in the pellet to the amount of radioactivity of a nonprecipitated sample. Aliquots of 100 ng/ml pBR322 containing 0, 50, 500, or 1000 µg/ml Torula Yeast RNA were ethanol precipitated and centrifuged for 5 min (solid diamond) or 30 min (solid box). Replicates of these samples were prepared in microfuge tubes obtained from West Coast Scientific (panels A and B), VWR Scientific (panel C), and Treff Lab (panel D).

Figure 2C. The recovery of DNA precipitated in one lot of microfuge tubes obtained from West Coast Scientific was increased when centrifugation was extended to 30 min (Figure 2B). However, when another lot of tubes from West Coast was tested , we did not observe this increase (Figure 2A). Microfuge tubes obtained from other manufacturers were also tested and the increase in recovery, if any, was slight (Figure 2C-D). Thus, extending centrifugation beyond 5 min is probably unnecessary in most cases.

Analysis of recovery of labeled pBR322 indicates that increasing the carrier RNA concentration from 50 to 500 µg/ml did not, in most cases, substantially improve recovery. However, at the higher concentration, the size of the nucleic acid pellet is such that it can easily be seen. This allows visual monitoring of the pellet which may prevent accidental loss in subsequent recovery steps.

The supernatants were also counted, and we consistently found that the radioactivity of the resuspended pellet plus the supernatant (total radioactivity recovered from the microfuge tube) was not identical in all samples (data not shown). Within each set of four tubes, the recovery of total radioactivity was lowest in samples containing 0-50 μg/ml RNA. This decrease in total radioactivity recovered was greatest in those tubes which had been centrifuged for 30 min. To determine if labeled DNA was adhering to the tubes, the tubes were subjected to a hot acid wash. The acidic solution was neutralized, and the isotope content of the tube was determined. We found that the tubes which contained low concentrations of RNA yielded the greatest number of counts adhering to the tubes and refractory to dissolution in buffer (data not shown). Because of this, percent recovery in the resuspended pellet is expressed as a fraction of radioactivity of a nonprecipitated sample rather than of the total radioactivity recovered.

We performed preliminary electron microscopic studies and found imperfections on the interior surface at the bottom of microfuge tubes. It is interesting to note that extended centrifugation markedly enhanced recovery in only one case; when a sample from that lot of tubes was examined, we found that the crevice-like flaws were minimal. It is possible that if the crevices are large enough, extended centrifugation may force the DNA aggregates into them making normal resuspension procedures insufficient.

ACKNOWLEDGMENTS

The authors wish to acknowledge helpful discussions with E.A. Birge, S.R. Szarek, and E.F. Robertson. We also thank W.P. Sharp for electron microscopic studies. This investigation received financial support from the Biomedical Sciences component of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases and by Public Health Service grant A120918 from the National Institute of Allergy and Infectious Diseases.

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