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Removal of RNase activity from DNase by affinity chromatography on agarose-coupled aminophenylphosphoryl-uridine-2'(3')-phosphate

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ABSTRACT

Severe degradation of high molecular weight RNA was shown to occur during incubation with commercially purified DNase. Most of the RNase activity could be removed by passage of the DNase through a column of agarose-coupled aminophenylphosphoryl-uridine-2'(3')-phosphate. Incubation with the treated DNase caused only minimal alteration of the sedimentation pattern of high molecular weight nuclear RNA, determined under partially denaturing conditions. No impairment of DNase activity was detected.

INTRODUCTION

It has been our experience, and that of other workers, that commercially available "RNase-free" DNase causes partial degradation of high molecular weight RNA. It has been reported that the RNase activity may be partly eliminated by DEAE-cellulose chromatography or by iodoacetate treatment(1). In our hands, these methods have failed to remove RNase activity adequately. Ribonuclease A from bovine pancreas has been purified by affinity chromatography involving adsorption to agarose-coupled aminophenylphosphoryl-uridine-2'(3')-phosphate (2), a material which is commercially available. We report here that RNase activity can be effectively removed from DNase by passage through a column of this derivatized agarose. This procedure is simple and rapid.

METHODS AND MATERIALS

Agarose: 5'-(p-aminophenylphosphoryl)-uridine-2'(3')-phosphate, prepared by Miles-Yeda Ltd., was obtained from Miles Laboratories, Inc., Elkhart, Ind. A column of this material (9 x 1 cm) was equilibrated with 0.02 M sodium

acetate, pH 5.2 (2). Deoxyribonuclease I (DPFF, Worthington Biochemical Corporation, Freehold, N.J.) was dissolved at a concentration of 20 mg/ml in the same buffer. Chromatography was carried out at room temperature. One ml of the DNase solution was applied to the column which was then eluted with 0.02 M sodium acetate, pH 5.2, during a period of 1.5 h. Fractions of 1 ml were collected and their absorbance at 280 nm was measured (Figure 1) before they were cooled on ice. Fractions 7 through 18 were pooled and stored frozen in small portions which were thawed only once before use.

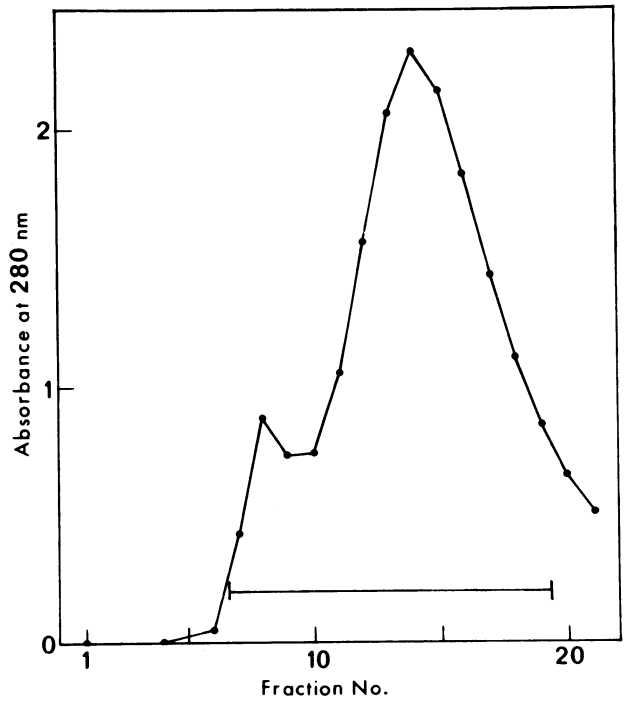

Figure 1. Chromatography of DNase on a column of agarose-coupled aminophenylphosphoryl-uridine-2'(3')-phosphate. 20 mg DNase was subjected to chromatography as described in Methods and Materials and the fractions indicated by the horizontal bar were then pooled.

Labeled RNA was extracted by a method avoiding the use of DNase (3, 4), from the nuclei of Ehrlich ascites tumor cells, which had been labeled with 2-^{14}C-uridine for 2.3 h in the presence of actinomycin D (0.05 µg/ml). Unlabeled RNA was extracted (5) from frozen cells of Escherichia coli K12.

Labeled DNA was extracted (4) from cultured mouse embryo fibroblasts which had been labeled for 3 days with [methyl-^{3}H] -thymidine. Unlabeled DNA from mouse liver nuclei was a gift from Dr. Sally Oklund.

Incubation mixtures (each in a volume of 1.0 ml) contained either untreated or treated DNase (0.094 absorbance units, equivalent to approximately 67 µg) or lacked DNase. The other components present were as follows: ^{14}C-RNA, 10,000 cpm (12 µg); unlabeled E. coli RNA, 90 µg; ^{3}H-DNA, 22,000 cpm (0.2 µg); unlabeled DNA, 3 µg; NaCl, 0.1 M; magnesium acetate,1 mM; tris-HCl, 10 mM, pH 7.3. The mixtures were incubated at 37°C for 30 min. Proteinase K (final concentration 50 µg/ml) was then added and incubation at 37°C was continued for 30 min. The mixtures were then deproteinized by extraction with phenol and chloroform in the presence of sodium dodecyl sulfate and the nucleic acids were precipitated from the aqueous phase by the addition of two volumes of ethanol.

Treatment of the recovered nucleic acids with dimethyl sulfoxide (80%,v/v) followed by sedimentation under partially denaturing conditions (5 - 20%, w/v, sucrose gradients in 50%, v/v, dimethyl sulfoxide, 0.1 M LiCl) was carried out as previously described (6), except that sodium dodecyl sulfate was omitted. The gradients were centrifuged in a Beckman SW 41 rotor for 15.5 h at 31,000 rpm and 20°C. HeLa nucleolar RNA (prepared as described by Penman (7)was centrifuged in a parallel gradient, to provide 32 S and 45 S markers. Fractions (0.37 ml) were collected, using ISCO equipment, starting from the top of the gradients. A sample (0.10 ml) of each fraction was mixed with a toluene-Triton scintillation cocktail and radioactivity was measured with a Beckman scintillation spectrometer, applying appropriate correction for channel overlap.

RESULTS AND DISCUSSION

RNase activity was assayed by the effect of incubation with DNase on the sedimentation profile of ^{14}C labeled heterogeneous nuclear RNA. Sedimentation analysis was conducted in sucrose gradients under partially denaturing condi-

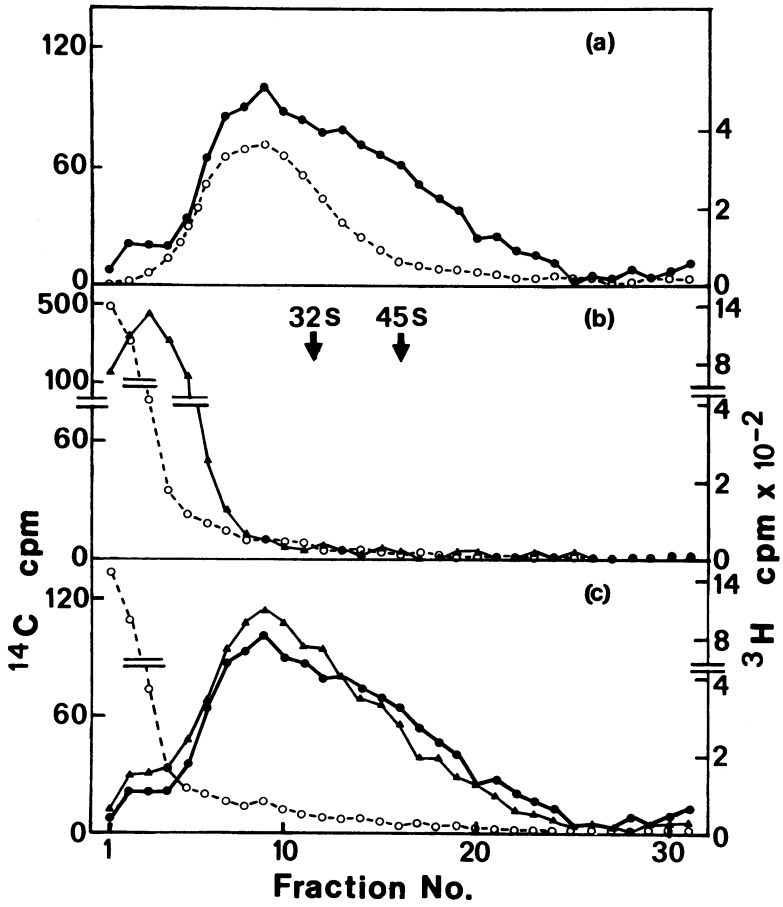

Figure 2. Sedimentation profiles in 50% dimethyl sulfoxide-sucrose gradients of ^{14}C-RNA and ^{3}H-DNA after incubation, (a) without DNase, (b) with untreated DNase, and (c) with DNase purified as shown in Figure 1. In (c), the ^{14}C-RNA profile of (a), above, is reproduced for direct comparison. The top of the gradients is shown on the left. ●——●, ^{14}C-RNA incubated without DNase. ▲——▲, ^{14}C-RNA incubated with DNase. O---O, ^{3}H-DNA.

tions. ^{3}H labeled DNA was included in all incubations in order to monitor DNase activity. As shown in Figure 2(a), labeled RNA, incubated in the absence of DNase, had a broad sedimentation profile comprised mainly of components between 16 S and about 60 S. Incubation in the presence of commercial "RNase-free" DNase resulted in most of the RNA sedimenting as a peak about 6 S and in the elimination of all RNA sedimenting faster than about 30 S (Figure 2(b)). The absorbance

pattern obtained from this gradient (not shown) indicated that severe degradation of unlabeled ribosomal RNA had also occurred.

The extensive degradation of ^{14}C-RNA, shown in Figure 2 (b), was essentially eliminated when ^{14}C-RNA was incubated with DNase which had been passed through the derivatized agarose (Figure 2(c)). Only a very slight effect on the sedimentation pattern of labeled RNA was observed and a substantial fraction of the ^{14}C-RNA sedimented faster than the 45 S marker. Under the denaturing conditions employed in the sedimentation analysis, the occurrence of only a few cleavages in high molecular weight RNA would be detected. This experiment therefore provided a very sensitive assay for residual RNase activity in the DNase.

In both Figures 2(b) and (c), the ^{3}H radioactivity (products of ^{3}H-DNA digestion) was present mainly near the top of the gradient, indicating that the DNase activity had not been impaired by passage through the column. Further support for this conclusion was obtained in another test using a larger quantity of DNA. In this test, high molecular weight DNA (100 µg/ml), incubated with the treated DNase under the same conditions as above (67 µg DNase per ml; 30 min at 37°C), was degraded to the extent that less than 1% of the DNA was excluded by Sephadex G-100 (results not shown). In contrast, high molecular weight RNA treated in the same way was excluded by Sephadex G-100.

As shown in Figure 2(c), the treated DNase retained a very slight RNase activity. This activity was not removed by a second passage through the column (results not shown) and may therefore have been due to contaminating traces of ribonucleases having substrate specificity different from ribonuclease A. Fairly rigorous incubation conditions were used in the experiments reported here: these conditions could probably be modified (shorter incubation time, lower DNase concentration, or lower temperature) to achieve adequate digestion of DNA with negligible effect on RNA.

The use of DNase in the isolation and purification of high molecular weight nuclear RNA has been hindered by the

presence of RNase activity in commercially available DNase. This consideration is especially important in studies of polyadenylated nuclear RNA. Significant cleavage of molecules in a population of polyadenylated RNA would result in an underestimate of the average molecular weight and in selective loss of sequences distant from the polyadenylated terminus if these molecules were subsequently isolated by methods such as oligo-(dT)-cellulose chromatography. It is difficult to obtain complete removal of DNA from nuclear RNA without the use of DNase. The DNase purification procedure described here promises to be of considerable value in providing DNase of adequate purity for use in the isolation of high molecular weight nuclear RNA.

Although partial removal of RNase from commercial DNase can be obtained by repeated chromatography on DEAE-cellulose ((1), and our unpublished observations), we have found the procedure reported here to be simpler and much more effective.

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