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Partial Purification and Characterization of Ribonuclease III Like Enzyme Activity from Cultured Mouse Embryo Cells†

G. Shanmugam‡

ABSTRACT: A ribonuclease with properties analogous to *Escherichia coli* RNase III has been partially purified from the cytoplasm of high-passage Swiss mouse embryo (HPME) cells. From 60 to 70% of the cytoplasmic enzyme activity (monitored using [^{3}H]poly(rA)·poly(rU) as substrate) was associated with the ribosomal and microsomal fractions and about 10% of the microsome-associated and 52% of ribosome-associated enzyme activity could be solubilized by 0.4 M KCl. The solubilized enzyme was further purified by ammonium sulfate fractionation and DEAE-cellulose (DE52) column chromatography. The DE52 enzyme degraded single-stranded ribopolynucleotides to an extent of 10-20% under the conditions that resulted in 80-100% solubilization of [^{3}H]poly(rA)·poly(rU). The cleavage of poly(rA)·poly(rU) was maximum at 25-50 mM K^+ and 2-5 mM Mg^{2+} ion concentrations and was inhibited by EDTA or *Penicillium chrysogenum* double-stranded (ds) RNA. The DE52 enzyme fraction showed contaminating hybrid nuclease activity but was devoid of DNase activity. Consistent with our previous observation with a microsome-associated nuclease from the same cell line, the partially purified enzyme endonucleolytically cleaved the polycistronic murine leukemia virus (MLV) 35S RNA. In addition, this enzyme preparation was capable of cleaving 45S ribosomal precursor RNA and the cleavage products had electrophoretic mobilities similar to those of mature ribosomal RNAs and their precursor intermediates.

Several different nucleases appear to be involved in the processing of cellular and viral RNAs (Perry, 1976; Robertson & Dickson, 1975). Ribosomal RNAs, mRNAs, and tRNAs are transcribed in the form of high-molecular-weight precursors that are later cleaved by specific nucleases to mature-size products. RNase III, an enzyme specific for double-stranded regions of RNA (Robertson et al., 1968), has been implicated in the processing of *Escherichia coli* rRNA precursors and bacteriophage T7 mRNA transcripts (Dunn & Studier, 1973). Another enzyme, RNase P, was shown to process tRNA precursors in *E. coli* (Robertson et al., 1972). Since RNA processing enzymes might be expected in eukaryotic cells due to the fact that they also contain RNA precursors which give rise to smaller mature RNAs, recent investigations have attempted to detect and study the role of these enzymes in the maturation of cellular RNAs (Rech et al., 1976; Ohtsuki et al., 1977; Hall & Crouch, 1977).

Our interest in RNA processing enzymes had an additional objective aimed at understanding the role of nucleases in the processing of polycistronic animal viral mRNAs. There appears to be precedence for a possible involvement of cytoplasmic nucleases in the processing of SV40 specific 19S mRNA to 16S mRNA in enucleated cells (Aloni et al., 1975). Therefore we initiated a search for eukaryotic nucleases that may be involved in the processing of viral RNAs (Shanmugam, 1976). These early studies indicated the presence of an RNase III like enzyme activity in the microsomal fraction of uninfected and oncornavirus infected cells which may be involved in the generation of the 20S virus-specific RNA species present in the microsomes (Shanmugam et al., 1974). Leis et al. (1978) recently identified a similar enzyme activity in duck embryo microsomes which cleaved Rous sarcoma virus 34S RNA. In both studies (Shanmugam, 1976; Leis et al., 1978) the microsome-associated particulate enzyme was used to cleave the oncornavirus RNAs. In studies described here, this ds RNase activity has been solubilized and partially purified from the cytoplasm of cultured mouse embryo cells. The partially purified enzyme has several properties (such as ribosomal association, ionic requirements, and preference for dsRNA) resembling *E. coli* RNase III and endonucleolytically cleaves nucleolar 45S ribosomal precursor RNA and polycistronic MLV RNA.

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0006-2960/78/0417-5052$01.00/0

Materials and Methods

HPME[1] cells grown in monolayer cultures (Shanmugam, 1976, 1977) were used for the preparation of nuclease and KB cells grown in suspension cultures (Shanmugam et al., 1975) were used for the preparation of 45S RNA and ribosomal marker RNAs. [^{3}H]Poly(rA)·poly(rU) (15.1 μCi/μmol of P), [^{3}H]poly(rU) (81.6 μCi/μmol of P), [^{3}H]poly(rC) (49 μCi/μmol of P), [^{3}H]poly(rA) (45 μCi/μmol of P), [^{3}H]poly(dA) (31.4 μCi/μmol of P), and [^{3}H]poly(dA)·(dT) (21.6 μCi/μmol of P) were obtained from Miles Laboratories, Inc. ^{3}H-labeled adenovirus DNA was prepared as described by Landgraf-Leurs & Green (1971). [^{3}H]Poly(rA)·poly(dT) (2000 cpm/pmol) and *Penicillium chrysogenum* viral RNA were kindly provided by Gary Gerard and Hugh D. Robertson, respectively.

Preparation of RNA. Nucleolar RNA was isolated by the method of Penman (1969) from 100 mL of KB cell suspension (6.6×10^8 cells) that were pulse-labeled for 15 min with 3 mCi of [^{3}H]uridine. The RNA was centrifuged in a gradient of 10–20% sucrose in $NaDodSO_4$ (Penman, 1969) at 36 000 rpm using a SW41 rotor. Aliquots of gradient fractions were counted, and fractions containing 45S RNA were pooled and precipitated with ethanol.

^{32}P-labeled MLV RNA was prepared from virus harvested at hourly intervals and heat dissociated at 70 °C for 2 min to obtain 35S RNA (Shanmugam, 1976).

Preparation of Nuclease. HPME cells were harvested using crushed frozen phosphate buffered saline (PBS) and washed twice with PBS. (All preparative procedures were done at 0–4 °C and the enzyme activity was followed by using [^{3}H]poly-(rA)·poly(rU) as substrate.) In a typical nuclease preparation, 8 mL (packed volume) of cells was swollen for 15 min in 3 volumes of reticulocyte standard buffer (10 mM Tris-HCl, pH 7.5 at 4 °C, 10 mM KCl, 1.5 mM $MgCl_2$) and homogenized, and nuclei were separated (Shanmugam, 1976). The salt concentrations of the postnuclear supernatant was adjusted to that of Tris buffer (50 mM Tris-HCl, pH 7.5 at 20 °C, 25 mM KCl, 5 mM $MgCl_2$, and 5% glycerol) and then treated with 0.4 M KCl to solubilize the enzyme. Microsomes from the KCl-treated cytoplasm were removed by centrifugation at 27 000 *g* for 10 min and the supernatant was centrifuged at 105 000 *g* for 2 h to sediment ribosomes. To the postribosomal supernatant, solid ammonium sulfate (30 g/100 mL) was slowly added and stirred at 4 °C for 20 min; the resulting precipitate was dissolved in 2 mL of Tris buffer containing 0.2 M KCl and dialyzed overnight in 2 L of the same buffer. The dialysate was applied to a DE52 column (1.5 × 20 cm) after tenfold dilution with Tris buffer. After adsorption, the column was washed with 180–200 mL of Tris buffer and then developed with a linear gradient of 0–0.6 M KCl in Tris buffer. Three-milliliter fractions were collected at a flow rate of 15 mL/h and 40-μL aliquots were assayed for enzyme activity.

Nuclease Assay. The synthetic polynucleotides and adenovirus DNAs were incubated in 0.1 mL of a standard reaction mix containing 20 mM Tris-HCl, pH 7.8 at 25 °C, 100 mM KCl, 5 mM $MgCl_2$, 5 mM β-mercaptoethanol, 5% glycerol, and the desired amounts of enzyme fractions. The incubations were done at 37 °C for the appropriate periods mentioned in the text. The reaction was stopped by the addition of 0.2 mL of cold 20% trichloroacetic acid in the presence of 200 μg of bovine serum albumin. Undigested material was centrifuged down and acid-soluble radioactivity in 0.2 mL of supernatant was determined as previously described (Shanmugam, 1976).

TABLE I: Assay of Soluble and Particle Associated Double-Stranded Ribonuclease Activities[a]

fractions	[^{3}H]poly(rA)·poly(rU) solubilized		
	cpm	pmol	%
microsomes (fraction A)	590	58.6	19.9
KCl-washed microsomes (fraction B)	528	52.5	17.8
microsomal KCl-wash (fraction C)	242	24.0	8.1
ribosomes (fraction D)	1060	106	35.9
postribosomal supernatant (fraction E)	1130	112	38.2
KCl-washed ribosomes (fraction F)	512	50.9	17.2
postribosomal supernatant from KCl-treated postmicrosomal supernatant (fraction G)	2570	256	86.8

[a] The cytoplasmic fractions described in Figure 1 were diluted 1:1 with Tris buffer, and 25-μL aliquots of the diluted fractions were incubated with 295 pmol (2964 cpm) of [^{3}H]poly(rA)·poly(rU) for 60 min and assayed for the release of acid-soluble radioactivity as described in Materials and Methods.

Nucleolar 45S RNA was incubated with the enzyme for 10 min under the conditions described above. At the end of incubation, the following reagents were added: 0.9 mL of Tris buffer without glycerol, 10 μL of ^{32}P-labeled cytoplasmic marker RNA, 50 μL (500 μg) of tRNA, 5 μL of diethyl pyrocarbonate, 3 mL of TNE (10 mM Tris-HCl, pH 7.4 at 4 °C, 0.1 M NaCl, 1 mM EDTA), 0.4 mL of 10% $NaDodSO_4$, 4.4 mL of 85% phenol, and 4.4 mL of $CHCl_3$–isoamyl alcohol (24:1). The mixture was shaken for 10 min and centrifuged. RNA was precipitated from the aqueous phase with 2 volumes of ethanol and electrophoresed as described below.

MLV 35S RNA was incubated for 10 min with the indicated amounts of enzyme preparation in 1-mL standard reaction mix containing 25 mM KCl. After incubation, the reagents mentioned above (except Tris buffer and [^{32}P]RNA) were added, and RNA was extracted and electrophoresed as described below.

Polyacrylamide Gel Electrophoresis. RNA samples were electrophoresed for 3–4 h in gels (0.6 × 10 cm) containing 2% acrylamide–0.1% bisacrylamide, and 0.5% agarose as described (Shanmugam, 1976; Shanmugam et al., 1974). The gels were fractionated into 2-mm fractions and counted in 10 mL of aquasol. In double-label experiments, the spill-over of ^{32}P into the ^{3}H channel was calculated with appropriate standards and the ^{3}H radioactivity corrected accordingly.

Results

In a previous study, ribosomal and microsomal fractions of HPME cells were shown to contain a nucleolytic activity which degraded [^{3}H]poly(rA)·poly(rU) (Shanmugam, 1976). The poly(rA)·poly(rU) cleavage activity is indeed a ds RNase since the degradation of this substrate was inhibited by ethidium bromide (Shanmugam, 1976) and *P. chrysogenum* ds RNA (see below). Therefore [^{3}H]poly(rA)·poly(rU) was used as substrate in experiments described below to monitor ds RNase activity.

Solubilization and Partial Purification of ds RNase. Table I shows the cytoplasmic distribution of ds RNase in different subcellular fractions prepared as shown in Figure 1 and the effect of high salt treatment on the release of this activity from particulate fractions. A quantitative comparison of the distribution of the enzyme in the subcellular fractions is not

[1] Abbreviations used: HPME, high-passage Swiss mouse embryo; MLV, Moloney murine leukemia virus; $NaDodSO_4$, sodium dodecyl sulfate; DEAE, diethylaminoethyl; EDTA, (ethylenedinitrilo)tetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; ds, double-stranded; ss, single-stranded; DTT, dithiothreitol; PBS, phosphate buffered saline.

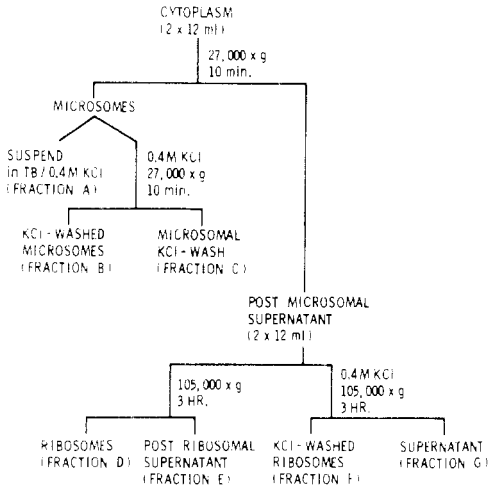

FIGURE 1: Schematic diagram of preparation procedures of particulate and soluble cytoplasmic fractions for ds RNase assays. Twenty four milliliters of post nuclear supernatant (cytoplasmic) fraction obtained as described in Materials and Methods was divided into two equal aliquots and centrifuged at 27 000 *g*. The microsomal pellet from one tube was suspended in 15 mL of Tris buffer (TB) containing 0.4 M KCl, while the pellet from the second tube was treated with 0.4 M KCl. The postmicrosomal supernatant was divided in the same manner and treated with KCl. Each particulate fraction was suspended in 15 mL of TB/0.4 M KCl and the soluble fractions were adjusted to 15 mL with the same buffer.

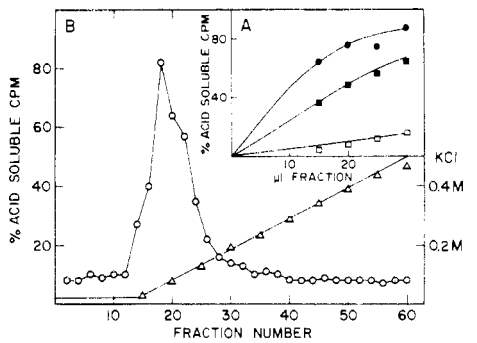

FIGURE 2: Partial purification of ds RNase. (A) $(NH_4)_2SO_4$ fractionation of proteins present in the 100 000 *g* supernatant of 0.4 M KCl treated cytoplasm. (●) A 0–15% cut; (■) 15–30% cut; (□) 30–40% cut. % refers to grams of solid $(NH_4)_2SO_4$ added to 100 mL of supernatant. Indicated aliquots of undiluted or tenfold diluted (■) samples were assayed for enzyme activity. (B) DE52 column chromatography of $(NH_4)_2SO_4$ fractionated enzyme. A 0–30% $(NH_4)_2SO_4$ cut prepared as above was dialyzed and then chromatographed. Forty-microliter aliquots of column fractions or the indicated amounts of $(NH_4)_2SO_4$ fractions were assayed for ds RNase activity using 436 pmol (4386 cpm) of [^{3}H]poly(rA)·poly(rU) as substrate under the conditions described in Table II.

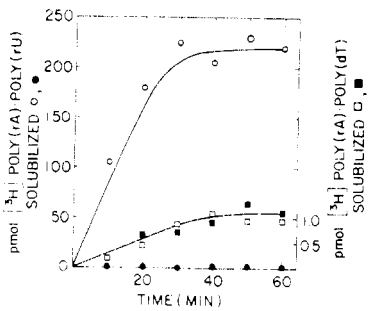

FIGURE 3: Inhibition of poly(rA)·poly(rU) cleavage activity by *P. chrysogenum* ds RNA. Constant amounts of DE52 enzyme preparation were incubated with 436 pmol (4386 cpm) of [^{3}H]poly(rA)·poly(rU) (○, ●) or 5.05 pmol (10 096 cpm) of [^{3}H]poly(rA)·poly(dT) (□, ■) in the presence (●, ■) or absence (○, □) of *P. chrysogenum* ds RNA (2 μg) in 100-μL incubation mixes and assayed for the release of acid-soluble radioactivity.

possible since the solubilized enzyme shows higher activity than the particle-associated enzyme. When aliquots of samples from subcellular fractions that were adjusted to equal volume were assayed for ds RNase activity, the nuclease was found to be distributed in both the microsomal and postmicrosomal supernatant fractions of HPME cells: in the latter fraction about 50% of the activity remained associated with ribosomes, and the rest was in the soluble postribosomal supernatant fraction (Table I). Part of the microsomal (10%) and a major proportion (52%) of the ribosome-associated ds RNase, as computed from the difference in enzyme activities remaining in the KCl-treated and untreated particulate fractions (Table I), could be solubilized by 0.4 M KCl. Further increase in KCl concentration to 0.6 M did not yield a significant increase in the solubilization of enzyme activity.

For partial purification of the enzyme, the cytoplasm of HPME cells was treated with 0.4 M KCl, the insoluble material removed by centrifugation, and the soluble supernatant was subjected to ammonium sulfate fractionation. A substantial quantity of ds RNase was precipitated by the addition of ammonium sulfate to 15% and maximum precipitation was observed by the addition of 15% more ammonium sulfate to the 0–15% supernatant (Figure 2A). Further addition of this salt did not result in a significant increase in the precipitation of enzyme activity (Figure 2A). Therefore, for routine enzyme purification, ammonium sulfate was added to the HPME cytoplasm to 30%. The resulting precipitate was dissolved in Tris buffer containing 0.2 M KCl, dialyzed, and applied to a DE52 column (see Materials and Methods). After absorption, the column was washed with 180–200 mL of Tris buffer. No poly(rA)·poly(rU) cleavage activity could be detected in the washings and all the ds RNase activity eluted from the column at a KCl concentration of 80–100 mM (Figure 2B).

Properties of Partially Purified Enzyme. The partially purified DE52 enzyme preferentially degraded [^{3}H]poly(rA)·poly(rU) (Table II) and this cleavage was completely inhibited by 20 μg/mL of *P. chrysogenum* ds RNA (Figure 3). The ds RNase activity was inactivated by heat (100 °C, 10 min) and inhibited by 20 mM EDTA. Maximum activity was observed in the presence of 25–50 mM Na^+, K^+, or NH_4^+ and 2–5 mM Mg^{2+} ions. Similar divalent cation concentrations of Mn^{2+} and higher concentrations of monovalent salts were inhibitory to enzyme activity. The cleavage of [^{3}H]poly(rA)·poly(rU) in the presence of 2 mM Mn^{2+} was 20% of that observed with 2–5 mM Mg^{2+} and the extents of inhibition of enzyme activity with increasing concentrations of KCl in comparison with the activity at 25–50 mM K^+ were: 100 mM, 30%; 150 mM, 49%; 200 mM, 65%; 300 mM, 86%; 400 mM, 95%.

As shown in Table II the mouse embryo ds RNase preparation also showed traces of RNase H and ss RNase activities. The hybrid nuclease activity appears to be due to the action of a contaminating nuclease rather than a property of ds RNase since the two activities showed different sedimentation profiles in sucrose gradients (Figure 4). Further, the hybrid nuclease activity was not inhibited by *P. chrysogenum* ds RNA (Figure 3). It is not clear, however, whether the ssRNase is another activity of the ds RNase or due to a contaminating enzyme. Double-stranded RNase preparations of calf thymus and chick embryo were found to have ss RNA degradation

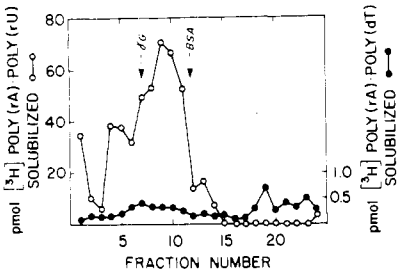

FIGURE 4: Sucrose density gradient centrifugation of DE52 enzyme. The DE52 enzyme pool was concentrated by ammonium sulfate (30%) precipitation and dialyzed against Tris buffer containing 0.3 M KCl. Two-hundred-microliter aliquots of this enzyme preparation or marker proteins (250 μg) in Tris buffer containing 0.3 M KCl were centrifuged in separate gradients of 5-20% sucrose in 50 mM Tris, pH 7.9, 50 mM KCl, 4 mM Mg^{2+} and 1 mM DTT for 24 h at 41 000 rpm in a SW40.1 rotor. The gradients were fractionated from the bottom and 25-μL aliquots of the fractions were used for enzyme assays. The gradient fractions were incubated for 30 min with either 436 pmol (4386 cpm) of [^{3}H]poly(rA)·poly(rU) or 5.05 pmol (10 096 cpm) of [^{3}H]poly(rA)·poly(dT) and assayed for the release of acid-soluble radioactivity. The amounts of acid-soluble radioactivities (cpm) obtained from enzyme assays for the peak fractions were: 877 cpm (fraction 9) with poly(rA)·poly(rU) substrate and 926 cpm (fraction 7) with poly(rA)·poly(dT) substrate.

TABLE II: Substrate Specificities of Mouse Embryo Nuclease[a]

substrate	input cpm	input pmol	solubilized cpm	solubilized pmol
[^{3}H]poly(rA)·poly(rU)	4 390	436	2040	203
[^{3}H]poly(rA)·poly(rU) and 20 mM EDTA	4 390	436	0	0
[^{3}H]poly(rU)	6 880	127	754	14
[^{3}H]poly (rU) and 20 mM EDTA	6 880	127	0	0
[^{3}H]poly(rA)	9 890	330	1270	43
[^{3}H]poly(rC)	5 740	176	795	24
[^{3}H]poly(rA)·poly(dT)	10 100	5.1	1580	0.8
[^{3}H]poly(dA)·poly(dT)	12 400	861	57	4
[^{3}H]adenovirus DNA (native)	3 680		0	
[^{3}H]adenovirus DNA (heat denatured)	2 640		0	

[a] The substrates and 40-μL aliquots of DE52 purified enzyme were incubated for 30 min at 37 °C in 100-μL standard reaction mixes and assayed for the release of acid-soluble radioactivity.

activities and no definite conclusion was drawn as to the origin of ss RNase activity (Hall & Crouch, 1977; Ohtsuki et al., 1977).

The DE52 enzyme was devoid of DNase activity as evidenced by a background level of solubilization of poly(dA)·poly(dT) and lack of degradation of adenovirus DNAs. However, the acid-solubilization assays are not sensitive enough to detect any nicks in DNA if produced by this enzyme.

Figure 4 also shows that the mouse embryo ds RNase activity sediments in sucrose density gradients close to γ-globulin (γG) with an estimated sedimentation coefficient of 6 S. Centrifugation in the presence of 0.4 M KCl in the gradient did not alter the sedimentation pattern. A ds RNase activity from Krebs ascites cells was found to elute close to γG in Sephadex G-200 chromatography (Rech et al., 1976). Since partially purified enzyme has been used in these studies, it is difficult to ascertain the molecular weight of ds RNase from the sedimentation or gel filtration data and an extensive purification of the enzyme is essential for the determination of the physical characteristics of the enzyme.

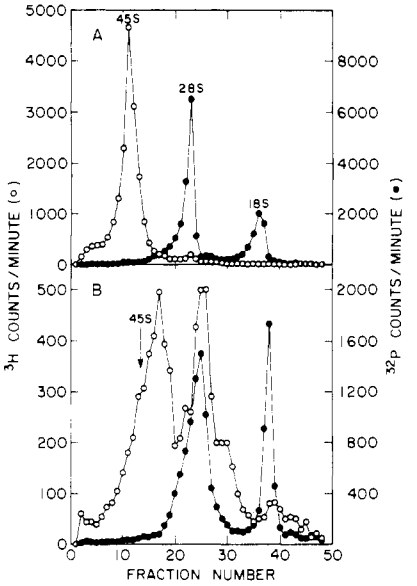

FIGURE 5: Cleavage of 45S nucleolar RNA by mouse embryo nuclease. [^{3}H]Uridine-labeled 45S ribosomal precursor RNA (140 000 cpm) was incubated for 10 min without (A) or with (B) 50 μL of DE52 enzyme. At the end of incubation, 150 000 cpm of ^{32}P-labeled cytoplasmic marker RNA was added to each sample, RNA reextracted and electrophoresed on 2% acrylamide-0.5% agarose gels.

Cleavage of 45S Nucleolar RNA. Since the mouse embryo nuclease showed several properties analogous to *E. coli* RNase III, it was of interest to know whether the mammalian enzyme could cleave 45S nucleolar ribosomal precursor RNA in a similar manner to the cleavage of 45S rRNA precursor by *E. coli* RNase III (Gotoh et al., 1974; Torelli et al., 1977). Therefore, the action of the mouse-embryo ds RNase on 45S nucleolar RNA isolated from pulse-labeled KB cells was determined. As shown in Figure 5, treatment of 45S RNA with 50 μL of the enzyme produced discrete cleavage products. The first major peak had a molecular weight of 3.1×10^6 similar to the 41S ribosomal precursor intermediates observed in in vivo studies (Weinberg & Penman, 1970). The second peak (molecular weight 1.65×10^6) migrated close to the 28S ribosomal marker RNA. Also minor peaks of cleavage products are seen in this figure; one of them migrates slightly faster than 18S marker RNA. *E. coli* RNase III was found to cleave HeLa cell 45S nucleolar RNA in a similar manner producing major cleavage products of 41S and 29S (Gotoh et al., 1974).

Cleavage of MLV 35S RNA. In agreement with previous observations with a microsome-associated ds RNase from the same cell line, the mouse embryo nuclease specifically cleaved the polycistronic mRNA obtained from MLV (Figure 6). The cleavage was inhibited by EDTA (Figure 6A) and ethidium bromide (Shanmugam, 1976) which is known to intercalate with double-stranded polynucleotides (Krugh & Reinhardt, 1975). The cleavage product had a molecular weight of approximately 2.2×10^6. In contrast, the microsome-associated enzyme from the same cell line produced a cleavage product of lower molecular weight, 1.8×10^6 from MLV 35S RNA (Shanmugam, 1976). Also the viral RNA (35S) showed lower electrophoretic mobility and higher molecular weight (3.7×10^6) in the presence of solubilized nuclease (Figure 6A) than that observed in the absence of enzyme or in the presence of particulate microsome-associated enzyme (Shanmugam, 1976). The lower electrophoretic mobility of the 35S RNA substrate and its cleavage products in the presence of solubi-

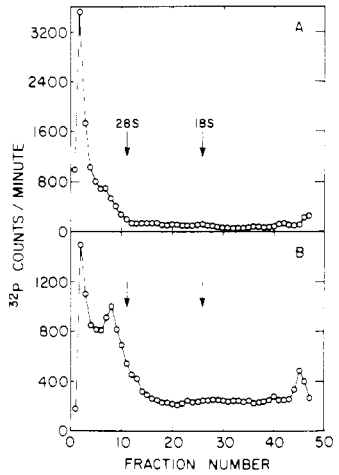

FIGURE 6: Cleavage of polycistronic MLV 35S RNA by mouse embryo nuclease. ^{32}P-labeled 35S RNA (45 000 cpm) was incubated for 10 min at 37 °C with 6 μL of DE52 enzyme in the presence (A) or absence (B) of 20 mM EDTA. At the end of incubation RNA was extracted from the incubation mixture and electrophoresed on 2% acrylamide–0.5% agarose gels. The arrows in the figure indicate the position of ^{32}P-labeled ribosomal marker RNAs that were electrophoresed in parallel gels.

lized enzyme may be due to a strong binding of the enzyme with these molecules. Such a binding may not occur with the particulate enzyme if the RNA binding sites were already associated with microsomes. EDTA appears to inhibit the enzyme action with no apparent effect on enzyme–substrate binding and therefore its use in the control experiment (Figure 6A) enabled the comparison of the electrophoretic mobilities of 35S RNA and its cleavage products under the conditions of their association with the enzyme.

Discussion

Several different ds RNase activities differing in physical and chemical properties were shown to be present in eukaryotic cells (Rech et al., 1976; Hall & Crouch, 1977; Ohtsuki et al., 1977; Shanmugam, 1976; Leis et al., 1978; Bothwell & Altman, 1975; Saha & Schlessinger, 1977; Birge & Schlessinger, 1974; Robertson & Mathews, 1973). These activities are widely distributed in various subcellular fractions. Nuclei of HeLa cells (Birge & Schlessinger, 1974; Saha & Schlessinger, 1977) and calf thymus (Ohtsuki et al., 1977) contain ds RNase capable of cleaving synthetic ds ribopolynucleotides and high-molecular-weight precursors to ribosomal and messenger RNAs. Cytosol and ribosomes of Krebs ascites cells show ds RNase activities which degrade poly(rC)·poly(rG) and bacteriophage f2 ds RNA (Rech et al., 1976; Robertson & Mathews, 1973). An endoplastic reticulum membrane fraction from KB cells was shown to possess ds RNase activity which degraded reovirus ds RNA (Bothwell & Altman, 1975). Recent studies indicate that microsomal fractions from mouse and duck embryo cells contain ds RNase activities capable of cleaving poly(rA)·poly(rU) and oncornavirus polycistronic mRNAs (Shanmugam, 1976; Leis et al., 1978).

The results presented in this paper demonstrate the existence of ds RNase in the cytoplasm of cultured mouse embryo cells. This nuclease had several properties such as ribosomal association, ionic requirements and ability to degrade ds RNAs, which resemble the well-known *E. coli* RNase III. The mouse embryo enzyme differs from *E. coli* RNase III in that it binds to DEAE and elutes at 80–100 mM KCl in the column chromatography (Figure 2), while the *E. coli* enzyme does not bind to DEAE. In this aspect, the mouse embryo nuclease resembles a chick embryo ds RNase termed RNase D II which was shown to have similar binding and elution characteristics (Hall & Crouch, 1977). Another ds RNase from calf thymus which binds more strongly to DEAE was also known to have properties resembling *E. coli* RNase III (Ohtsuki et al., 1977). The calf thymus enzyme cleaves 45S nucleolar RNA producing a heterogeneous population of cleavage products while the mouse embryo enzyme described in this study produces discrete cleavage products from 45S RNA (Figure 5). In addition, the mouse embryo enzyme was shown to cleave the polycistronic mRNA of MLV. These results confirm our previous observations and those of Leis et al. (1978) in which the microsomes associated particulate enzyme was used to cleave oncornavirus RNAs.

E. coli RNase III was known to cleave 45S nucleolar (Birge & Schlessinger, 1974; Torelli et al., 1977) and oncornavirus 35S RNA (Leis et al., 1978); the cleavage products had molecular weights comparable to those produced by the mouse embryo enzyme. However, only a comparison of fingerprints of RNase digests or some other sequence-related assay of the cleavage products will allow a more definite conclusion on the similarities or differences of the cleavage products produced by the prokaryotic or eukaryotic ds RNases.

The action of the mouse embryo enzyme on the polycistronic animal viral mRNA may be analogous to the cleavage of bacteriophage T3 and T7 mRNA transcripts by *E. coli* RNase III (Dunn & Studier, 1973). If such a cleavage is mediated by the eukaryotic enzyme in vivo, this may have important implications on the regulation of oncornavirus replication and a detailed structural and functional characterization of the cleavage products could provide information on the in vivo role of this enzyme in the generation of functionally active small molecular weight RNAs from larger mRNAs. For such studies, oncornavirus RNAs would prove useful since these are polycistronic animal virus mRNAs containing well-defined regions coding for specific classes of proteins (Baltimore, 1974).

Acknowledgments

I thank Max Arens, Gary Gerard, Hugh Robertson, and David Schlessinger for critical comments on the manuscript and Nancy Schulte for excellent typing. I thank Dr. Robertson also for encouragement and suggestions during the course of this study.

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Structural Limitations on the Bifunctional Intercalation of Diacridines into DNA†

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ABSTRACT: An homologous series of diacridines containing two 9-aminoacridine chromophores linked via a simple methylene chain has been studied in order to investigate the minimum interchromophore separation required to permit bifunctional intercalation. Viscometric, sedimentation, and electric dichroism experiments show that compounds having one to four methylene groups in the linker are restricted to monofunctional intercalation, whereas the interaction becomes bifunctional when the chain length is increased to six carbons or more. The results indicate that bifunctional reaction occurs with an interchromophore distance not exceeding 8.8 Å, implying that intercalation by these compounds is not subject to neighbor exclusion if the mode of binding is of the classical intercalation type.

The present study stems from a convergence of several recent advances in our understanding of drug–nucleic acid interaction: the characterization of quinoxaline antibiotics as bifunctional DNA-intercalating agents (Waring & Wakelin, 1974; Wakelin & Waring, 1976), the synthesis of diacridines as a possible route to develop compounds having enhanced binding affinity as well as possible additional specificity (Canellakis et al., 1976a–c), and the use of electric dichroism as a probe for geometrical relationships between ligands bound to DNA and the DNA base pairs (Houssier & Fredericq, 1966; Houssier et al., 1974). The possibility of using diacridines having potentially intercalative chromophores linked by a simple, flexible methylene chain of varying length to investigate the minimum separation between chromophores needed to permit bifunctional interaction has already been suggested (Canellakis et al., 1976a). Here we report an investigation into the interaction between such a series of diacridines and DNA, employing (1) sedimentation and viscometric measurements on closed circular duplex DNA from bacteriophage PM2 to study relative helix-unwinding angles, (2) viscometric observations on sonicated rodlike fragments of DNA to determine helix extension parameters, and (3) electric dichroism measurements designed to yield information concerning the relative orientations of the acridine chromophores with respect to the DNA base pairs. The compounds studied constitute an homologous series in which two 9-aminoacridine rings are joined via their amino groups with a simple methylene chain. For purposes of comparison, some experiments were also performed using the simple intercalating agents 9-aminoacridine and 9-methylaminoacridine.

Materials and Methods

Structural formulas of the acridine derivatives, together with their respective maximum 9-amino nitrogen/9′-amino nitrogen distances and their visible and ultraviolet extinction coefficients, are given in Table I. Also included are the hitherto

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0006-2960/78/0417-5057$01.00/0