

An Extracellular Nuclease from *Serratia marcescens*

II. SPECIFICITY OF THE ENZYME*

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SUMMARY

The specificity of an extracellular nuclease from *Serratia marcescens* was examined with both RNA and DNA as substrates. The enzyme was found to act in a predominantly endonucleolytic manner with less than 2% of the degradation products being mononucleotides. Both DNA and RNA were degraded to di-, tri-, and tetranucleotides terminating in 5'-phosphoryl ends. The enzyme had the unusual property of hydrolyzing both single-stranded and double-stranded DNA and RNA at similar rates.

No base preference was exhibited at either the 5' or 3' end of the oligonucleotide fragments. When the *S. marcescens* nuclease was used in conjunction with venom phosphodiesterase, RNA and DNA were rapidly degraded to 5'-mononucleotides. The enzyme is thus potentially useful as a reagent for the study of polynucleotide structure and sequence.

In an accompanying paper, the purification and some of the properties of an extracellular nuclease from *Serratia marcescens* were described (1). This report deals with the mode of attack of this enzyme on RNA and DNA substrates. The investigations were undertaken with the hope that the enzyme would prove to possess an interesting base specificity. Although no such specificity was found, the *S. marcescens* nuclease described here does produce oligonucleotides of defined size; the products of nucleolytic digestion of both DNA and RNA are di-, tri-, and tetranucleotides terminating in a 5'-phosphate. The specific size of the products, in addition to other properties of the enzyme, might permit its use as a reagent for the study of poly-

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nucleotide structure and sequence, particularly with oligonucleotides of moderate size.

EXPERIMENTAL PROCEDURE

Materials

All RNase assays were performed using *Torula* RNA (B grade) which was purchased from Calbiochem. For determination of the products of nuclease digestion, a high molecular weight preparation of cytoplasmic RNA from Ehrlich ascites tumor cells was used. This material was prepared both as nonradioactive RNA and as ³²P-labeled RNA according to the procedure of Roberts, Newman, and Rueckert (2).

DNase assays and digestions were performed with either calf thymus DNA (A grade) or salmon sperm DNA (A grade) obtained from Calbiochem. Denatured DNA was prepared from native calf thymus DNA by heating at 100° for 15 min, then cooling quickly by immersion in an ice bath. The hyperchromicity of the denatured DNA solution was 22%.

The DEAE-Sephadex Fraction 7 of the *S. marcescens* nuclease was used in all experiments to be described. Venom phosphodiesterase, intestinal alkaline phosphatase, and bacterial alkaline phosphatase were products of Worthington.

DEAE-cellulose (Selectacel) was a product of the Schleicher and Schuell Company, and was prepared in a manner similar to the method of Peterson and Sober (3). It was thoroughly washed with distilled water by decantation and then equilibrated 15 min with 0.5 M NaOH. The solution was neutralized, acidified with 1 M HCl, and washed with water on a Buchner funnel until the effluent pH was nearly neutral. The solution was equilibrated 15 min with 1 M NaOH, and again filtered and washed with water. The DEAE-cellulose was stored in 1 mM EDTA, pH 7.0.

Reference nucleotide and nucleoside standards were products of Calbiochem. Buffers and chemicals not otherwise specified were of reagent grade. The standard assay buffer was 50 mM Tris-HCl pH 8.2, 1 mM MgCl₂.

Methods

Enzyme Assays—Assays of the *S. marcescens* nuclease with RNA and native and denatured DNA substrates were performed as described in the previous paper (1).

Analysis of Products of Digestion—DEAE-cellulose chromatography with 7 M urea in the elution buffers was performed in a

manner similar to the method described by Tomlinson and Tener (4). Specific conditions are discussed in the legends to Figs. 1 and 4. Concentrated urea solutions were deionized by filtration through a mixed-bed resin (Bio-Rad AG 501-X8) before incorporation into the elution buffers.

The fractions eluted from DEAE-cellulose columns were desalted by the method of Rushizky and Sober (5). Combined column fractions were diluted 100-fold with water, and applied to a column (1.2 × 5 cm) of DEAE-cellulose which had been previously equilibrated with 1 mM ammonium carbonate. The column was washed with about 500 ml of 1 mM ammonium carbonate, and then eluted with 0.7 to 1.0 M ammonium carbonate. The fractions of highest absorbance at 260 m μ (10 to 15 ml total) were combined and repeatedly flash evaporated (8 to 12 times) until there was no further decrease in visible salt. The desalted sample was washed from the flask in a total of 2 ml of water and was air evaporated over a 37° water bath to a very small volume (0.1 to 0.3 ml) appropriate for chromatography or further treatment.

Paper electrophoresis was performed at 900 volts with Whatman No. 3MM (W. and R. Balston, Ltd., London, England) papers (57 × 15 cm) with the sample applied 10 cm from one end.

The eight common ribonucleosides and ribonucleotides were separated by paper chromatography as described by Nestle and Roberts (6), with paper previously treated with sodium phosphate and an isopropanol-ammonia-water (7:1:2) solvent system. (It was subsequently found that changing the solvent proportions to 7:1:3 gave a better and more rapid separation, particularly of the ribonucleotides.) This method gave excellent separation of the ribonucleotides and ribonucleosides, but it did not resolve the corresponding deoxy compounds. Thymidylic acid was only partially separated from deoxycytidylic acid, and thymidine and deoxyadenosine moved as a single spot.

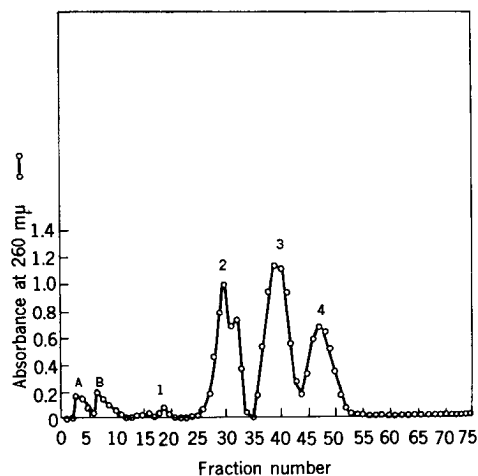


FIG. 1. DEAE-cellulose chromatography of an *S. marcescens* nuclease digest of RNA in the presence of urea. The 1.2-ml digestion mixture contained 5.5 mg of ascites tumor cell cytoplasmic RNA and 0.4 ml of enzyme in 0.1 M Tris-HCl, pH 8.2, 0.01 M MgCl₂. After 32 hours at 37°, the digest was mixed with 1.2 ml of 0.02 M Tris-HCl, pH 7.5, containing 7 M urea (urea-buffer), and applied to a column (1 × 25 cm) of DEAE-cellulose which had been equilibrated with the urea-buffer. The column was eluted with a gradient consisting of 500 ml of urea-buffer in the mixing chamber and 500 ml of 0.4 M NaCl in the buffer in the reservoir. Fractions (9 ml) were collected at a flow rate of 1 ml per min, and their absorbance was read at 260 m μ . Identification of the resulting peaks is in the text.

Quantitative elution of ultraviolet-absorbing spots was accomplished by either of two methods. (a) Small spots were cut out of the chromatogram, cut into small pieces, and suspended in 0.1 M HCl for 2 to 4 hours at room temperature with occasional shaking. A spot of equal size, preferably from the same area of the paper, was used as a blank for each sample. The samples were centrifuged briefly and the supernatant fluids were used for further study. (b) Long strips were eluted by descending chromatography, usually with water. The 1 to 2-ml eluent was concentrated by air evaporation for further use.

Venom phosphodiesterase hydrolysates were prepared for chromatography as follows. Aliquots (0.1 ml) of *S. marcescens* nuclease digests of RNA or DNA were mixed with 0.05 ml of a 1 mg per ml solution of venom phosphodiesterase dissolved in 0.1 M Tris-HCl, pH 9.0, and incubated 60 min at 37°. The digestion mixture could be spotted directly on the paper previously treated with sodium phosphate.

Digests were prepared for electrophoresis as described above except that they were all performed in 0.1 M potassium carbonate, pH 8.8, 0.01 M MgCl₂. The potassium carbonate and protein were removed from solution by lowering the pH of the digestion mixture to pH 3 to 4 with 12% perchloric acid. After centrifugation, the supernatant fluid could be directly applied to the paper.

Additional experiments were performed as described in the previous paper (1).

RESULTS

The specificity of the purified *S. marcescens* nuclease was determined on both RNA and DNA substrates in terms of size of products formed, position of terminal phosphoryl group, and possible base specificity. The minimum size required for an oligonucleotide to serve as substrate was established, and a comparison was made of enzymatic activity toward native and denatured DNA and single and double-stranded RNA.

Activity of *S. marcescens* Nuclease toward RNA

Size of Products Formed—The products of a prolonged *S. marcescens* nuclease digestion of high molecular weight RNA were analyzed by chromatography on DEAE-cellulose as illustrated in Fig. 1. Six peaks of material absorbing at 260 m μ were found which accounted for essentially all of the original absorbance. The first two small peaks (A and B) had absorption spectra which indicated that they were not nucleic acids. The spectrum of Peak A showed end absorption with no maximum, while that of Peak B showed maximum absorption around 280 m μ . Peaks 1 to 4, which had spectra with maxima around 260 m μ , were identified as mono- to tetranucleotides by (a) cochromatography with a pancreatic ribonuclease digest of ³²P-labeled RNA, and (b) chromatographic analysis after alkaline hydrolysis as described below. The mononucleotide region represented less than 2% of the total absorbance at 260 m μ , and with limit digests of high molecular weight RNA there was no absorbance in fractions corresponding to penta- or higher nucleotides. Chromatography of the products of shorter digestions, or of the commercial *Torula* RNA, showed small peaks in the higher oligonucleotide positions. The results of this experiment indicate that under optimal conditions the *S. marcescens* nuclease completely digests RNA to tetra-, tri-, and dinucleotides, together with a very small fraction of mononucleotides.

The products from an earlier stage of digestion of RNA are

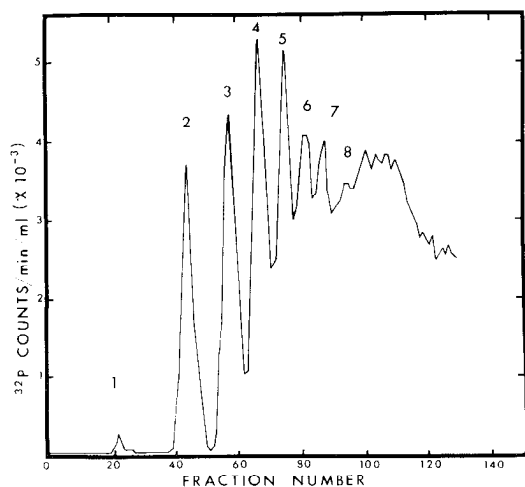


FIG. 2. DEAE-cellulose chromatography of a *S. marcescens* nuclease partial digest of RNA. Ascites cell RNA (1.5 mg, containing 1.2×10^6 cpm of ^{32}P) was dissolved in 2.0 ml of 0.05 M Tris-HCl, pH 8.2, 0.001 M MgCl_2 , 20 units of nuclease were added, and the solution was incubated at 37° for 20 min. The digest was heated at 95° for 2 min to stop the reaction, and then it was placed on a DEAE-cellulose column (1.2 \times 12 cm) which had been equilibrated with the urea buffer. Elution was with 250 ml of urea-buffer-0.4 M NaCl passing into 250 ml of urea-buffer; the flow rate was 0.3 ml per min, and 3.5-ml fractions were collected. Aliquots from each fraction were plated and counted in a gas flow counter.

shown in Fig. 2. Here, high molecular weight ^{32}P -labeled RNA was hydrolyzed for 20 min with 20 units of *S. marcescens* nuclease. Precipitation of an aliquot of the reaction mixture with perchloric acid under the standard assay conditions showed 55% of the radioactivity to have been solubilized by this treatment. It is clear from Fig. 2 that the limited hydrolysis, in addition to di-, tri-, and tetranucleotides, has released large amounts of penta-, hexa-, hepta-, and higher oligonucleotides from the RNA (20% of the added radioactivity was not recovered from the column during this elution). This suggests that the *S. marcescens* nuclease attacks RNA in an endonucleolytic manner producing large oligonucleotides which are further hydrolyzed to yield di-, tri-, and tetranucleotides as the final, nuclease-resistant products of a complete digestion. It is not known whether the radioactivity in the mononucleotide region represents enzymatic release of a small amount of mononucleotide or a radioactive contaminant in the RNA.

Minimum Size of Digestible Substrate—A series of experiments was performed to determine the minimum size of oligonucleotide hydrolyzable by the *S. marcescens* nuclease. The tri-, tetra-, and pentanucleotide fractions isolated from a limited digestion of ^{32}P -labeled RNA were subjected to further prolonged hydrolysis by the *S. marcescens* nuclease. The products of each fraction were then chromatographed along with a digest of unlabeled RNA on DEAE-cellulose in the presence of urea. As shown in Fig. 3, there was very little, if any, hydrolysis of the tri- and tetranucleotide substrates. The pentanucleotides, however, were almost completely degraded. The products can be seen to be primarily di- and trinucleotides. It was concluded that the *S. marcescens* nuclease cannot effectively hydrolyze an RNA substrate smaller than a pentanucleotide.

Position of Terminal Phosphoryl Group—The location of the terminal phosphate group on the products of enzymatic hydrolysis was determined by treating *S. marcescens* nuclease digests of

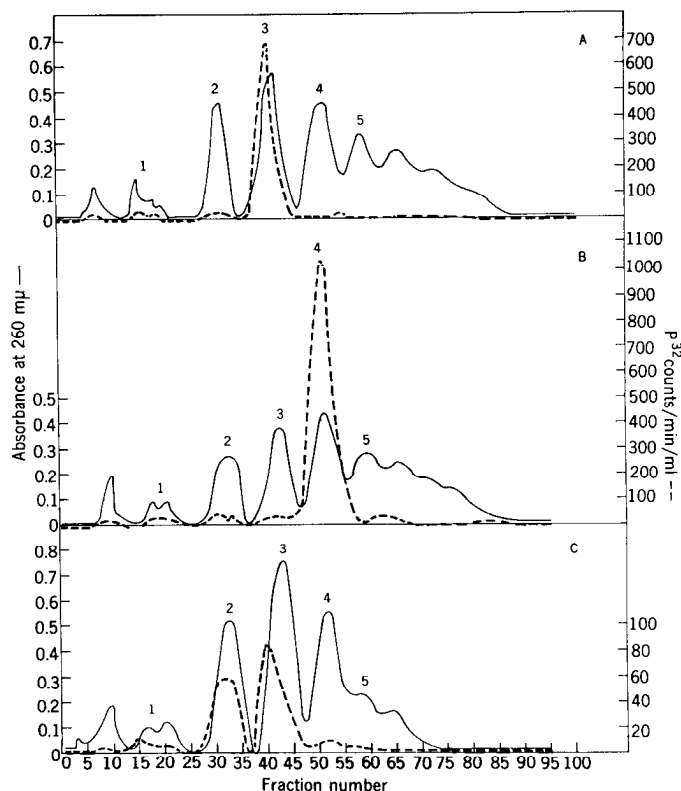


FIG. 3. Minimum size of ribonucleotide substrate hydrolyzable by the *S. marcescens* nuclease. Labeled substrates were prepared from an *S. marcescens* nuclease digest (18 hours at 37°) of ^{32}P -labeled Ehrlich ascites tumor cell cytoplasmic RNA which had been chromatographed on DEAE-cellulose in the presence of 7 M urea as described in the legend to Fig. 1. Fractions comprising the various peaks were combined and desalted as described under "Methods." The substrates were brought to 1-ml volumes with 0.1 M Tris-HCl, pH 8.2, 0.01 M MgCl_2 , and digested for 48 hours at 37° with a total of 100 enzyme units. The same amount of enzyme was also used to digest a solution of 5 mg per ml of *Torula* RNA for 24 hours. The two digests were combined and rechromatographed on DEAE-cellulose in the presence of 7 M urea as usual. 1 through 5, peaks corresponding to mono- to pentanucleotides. A, trinucleotide substrate; B, tetranucleotide substrate; C, pentanucleotide substrate.

RNA or DNA with venom phosphodiesterase and separating the digestion products by chromatography or electrophoresis as described under "Experimental Procedure." Venom phosphodiesterase cleaves 3'-hydroxyl-terminated ribo- or deoxyribo-oligonucleotides in an exonucleolytic manner from the 3' terminus such that 5'-mononucleotides are produced (7). Cleavage of oligonucleotides terminating in 3'-phosphate, however, would occur very slowly, and would leave three products: nucleoside 3', 5'-diphosphates, nucleotides terminating in 5'-phosphate, and nucleosides.

All products resulting from venom phosphodiesterase treatment of *S. marcescens* nuclease digests of RNA and DNA chromatographed solely in the nucleotide region. Under the conditions described, there was little ultraviolet-absorbing material left near the origin (less than 5%) where nucleoside diphosphates would be expected to be located. There were no ultraviolet-absorbing spots in the nucleoside region. It was thus concluded that the phosphoryl group is located at the 5' terminus. This result was confirmed by the electrophoretic separation of the digestion products.

TABLE I

Identification of dinucleotide sequences from an *S. marcescens* nuclease digest of RNA

The fractions comprising the dinucleotides from a DEAE-cellulose column eluted in buffers containing urea (see Fig. 1) were combined and desalted as described under "Experimental Procedure." The sample was hydrolyzed with 0.4 M KOH for 18 hours at 37°, then neutralized with 12% perchloric acid and subjected to electrophoresis in 0.02 M ammonium acetate buffer pH 5.8 for 2 hours. This procedure separates the nucleoside diphosphates (5' terminus) from the nucleosides (3' terminus) which remain at the origin. The spots were eluted with water and the eluent was concentrated to about 0.1 ml. The nucleoside diphosphates were dephosphorylated with intestinal alkaline phosphatase (1 mg in 0.2 ml 0.2 M glycine buffer, pH 9.5) for 2 hours at 37°. Both samples were chromatographed on paper previously treated with sodium phosphate as described under "Methods." The ultraviolet-absorbing spots were cut out and eluted in 2 ml of 0.01 N HCl and their spectra were determined. The molar amounts of the nucleosides were calculated from the spectral data. Total micromoles: 5' terminus = 0.44, 3' terminus = 0.39. Ratio of 5' terminus to 3' terminus = 1.1.

Base	Nucleoside found at terminus	
	5' Terminus	3' Terminus
	%	
Guanosine.....	22.0	34.4
Uridine.....	41.4	18.6
Cytidine.....	15.8	23.4
Adenosine.....	20.8	23.6

Base Specificity—An absolute base specificity of the *S. marcescens* nuclease for RNA would seem unlikely because of the virtual absence of mononucleotides or oligonucleotides larger than tetranucleotides among the digestion products. Nevertheless, the di-, tri-, and tetranucleotide peaks from a DEAE-cellulose column were examined for the possible absence of one or more nucleotides at either the 3'- or 5'-linked ends.

The dinucleotide fraction from a DEAE-cellulose column was subjected to alkaline hydrolysis, and the products were analyzed by chromatography on DEAE-cellulose as described in the legend to Table I. The results of a typical experiment are presented in Table I. No base preference was found at either the 5' terminus (represented by nucleoside diphosphates) or the 3' terminus (nucleosides). Although the exact distribution of bases was found to vary from experiment to experiment, the general lack of base preference was typical.

The tri- and tetranucleotide fractions were treated in the same manner as the dinucleotides. No base preference was found at either the 3'- or 5'-ends, in agreement with the analysis of dinucleotide sequences. The evidence from these experiments is consistent with the idea that the *S. marcescens* nuclease lacks absolute base specificity on RNA substrates.

Activity toward Double-stranded RNA—The ability of *S. marcescens* nuclease to hydrolyze a double-stranded polyribonucleotide was tested with poly I·poly C.¹ The rate at which this polymer was solubilized, using either *Serratia* nuclease or pancreatic ribonuclease as a control, is shown in Fig. 4. The poly I·poly C was completely resistant to hydrolysis by pancreatic

¹ The abbreviations used are: poly I, polyinosinic acid; poly C, polycytidylic acid.

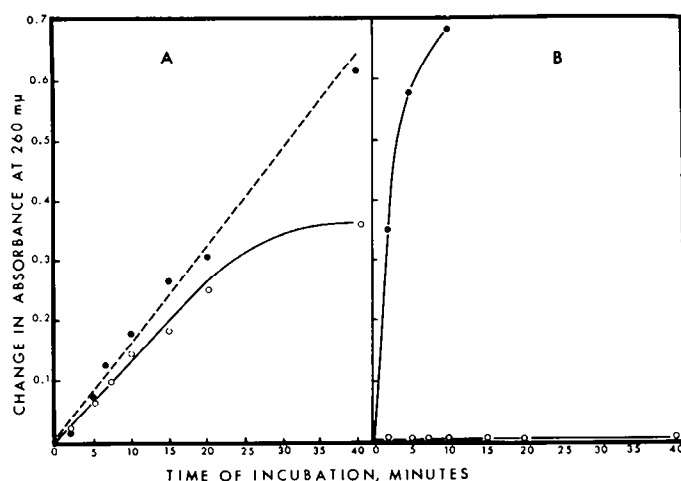


FIG. 4. Rate of hydrolysis of poly I·poly C with *S. marcescens* nuclease and pancreatic ribonuclease. Incubations were at 37°, and all assays were performed by removing 0.2 ml from the incubation mixture at the times indicated and assaying for acid-soluble absorbance as previously described. Experiment A contained 4 units of *S. marcescens* nuclease, and either 0.7 mg of the double-stranded poly I·poly C (P-L Biochemicals), or 1.2 mg of *Torula* RNA dissolved in 1.8 ml of buffer (0.1 M Tris-HCl, pH 8.4, 0.001 M MgCl₂). Experiment B contained 20 units of pancreatic ribonuclease and either 0.7 mg of poly I·poly C or 1.2 mg of *Torula* RNA dissolved in 1.8 ml of buffer (0.1 M Tris-HCl, pH 7.6, 0.001 M MgCl₂). ○, incubations with poly I·poly C; ●, incubations with *Torula* RNA.

ribonuclease. The *S. marcescens* nuclease, however, hydrolyzed the double-stranded polymer at a rate similar to that of single-stranded *Torula* RNA.

The activity of *S. marcescens* nuclease toward double-stranded RNA was investigated further by using the replicative form of viral RNA found in Ehrlich ascites cells following infection with ME virus (2) as a "natural" double-stranded RNA. Virus-specific RNA can readily be detected in this system by labeling infected cells with a radioactive RNA precursor in the presence of actinomycin D (which specifically inhibits the cellular RNA synthesis). The radioactive, double-stranded, replicative form can then be distinguished from the radioactive, single-stranded, viral RNA by virtue of its relative resistance to hydrolysis by pancreatic ribonuclease.

Fig. 5 shows the sedimentation profiles of virus-specific RNA before and after nuclease treatment. The closed circles represent the sedimentation of viral RNA before nuclease treatment. The two main peaks correspond to the typical 35 S peak of single-stranded viral RNA and a peak of replicative intermediate at about 20 S (2). The arrows show the sedimentation of the 28 S and 18 S ribosomal RNA in this sample determined by optical density measurements.

Treatment of this RNA with pancreatic RNase and pancreatic DNase resulted in the pattern indicated by the open triangles. This treatment shifted all optical density and most of the radioactivity to the top of the gradient leaving, at about 20 S, a distinct peak of RNase-resistant, double-stranded RNA. This RNA has previously been shown to be infectious (2). In contrast, treatment of the RNA with the *S. marcescens* nuclease (open squares) resulted in essentially all of the optical density and radioactivity, including the peak of double-stranded RNA at 20 S, being shifted to the top of the gradient.

This difference in the abilities of pancreatic RNase and *S. marcescens* nuclease to degrade double-stranded RNA was observed even after a 10-fold increase in the amount of pancreatic RNase and a 10-fold decrease in the amount of *S. marcescens* nuclease. The results in Fig. 5 were obtained using 200 units (about 1 μ g) of pancreatic RNase and 40 units of *S. marcescens* nuclease. Increasing the amount of pancreatic RNase to 2000 units still gave a sedimentation pattern very similar to the open triangles in Fig. 5. Decreasing the amount of *S. marcescens* nuclease to 4 units left a small amount of ribosomal and viral RNA which was degraded but still sedimentable. However, no trace of a peak could be detected at 20 S and, in fact, less radioactivity was found in this region than was found following the

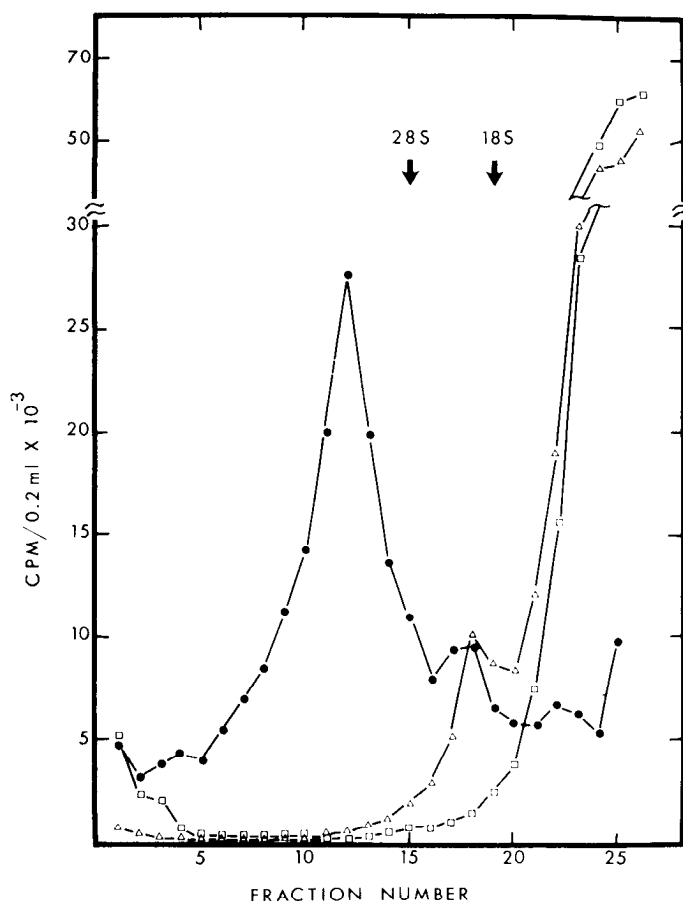


FIG. 5. Effect of nuclease treatment on the sedimentation of viral RNA. Ehrlich ascites cells were infected with ME virus and labeled with 32 P for 8 hours in the presence of 5 μ g per ml of actinomycin D as previously described (2). The cells were fractionated, and the RNA from the total cytoplasmic fraction was extracted (2). Samples of cytoplasmic RNA, equivalent to that obtained from about 0.1 g of cells, were treated as follows. Sample was dissolved in 1.0 ml of gradient buffer and centrifuged without further treatment (●—●); sample was dissolved in 1.0 ml of 0.1 M Tris-HCl, pH 7.6, 0.01 M $MgCl_2$, 200 units of pancreatic RNase and 5 μ g of pancreatic DNase were added, the solution was incubated 15 min at 37°, and then centrifuged (△—△); sample was dissolved in 1.0 ml of 0.1 M Tris-HCl, pH 8.4, 0.01 M $MgCl_2$ and 40 units of *S. marcescens* nuclease were added, the solution was incubated 15 min at 37°, and then centrifuged (□—□). The zonal centrifugation was performed in a 5 to 20% sucrose gradient as previously described (2). One-milliliter fractions were collected and 0.2-ml aliquots were plated and counted in a Nuclear-Chicago gas flow counter.

pancreatic RNase treatment. Therefore, it was concluded that *S. marcescens* nuclease hydrolyzes single- and double-stranded RNA at similar rates.

Activity of *S. marcescens* Nuclease toward DNA

Activity toward Native and Denatured DNA—The time course of hydrolysis of native and denatured calf thymus DNA is given in Fig. 6. Similar curves were obtained with native and denatured λ bacteriophage DNA substrates. The slight sigmoidal shape of the curves may reflect the fact that initial endonucleolytic attack of a high molecular weight substrate could be expected to yield many acid insoluble products, whereas at later stages of hydrolysis the products would be primarily acid soluble.

The initial rate of hydrolysis was slightly greater on denatured than on native DNA, but after a 25-min incubation, when nearly 50% of the substrate had been converted to acid-soluble material, the rates were equal. The rate on native DNA then became slightly higher with 95% of the native DNA and 80% of the denatured substrate being rendered acid-soluble. This effect may be due to a slight preference of the enzyme for denatured or partially denatured region of DNA. In general, however, the effect is a small one, and the enzyme seems to act with essentially equal efficiency on both substrates.

Activity toward Native DNA

Size of Products Formed—The products of a digest of DNA were analyzed in the same manner as those from an RNA digest. As shown in Fig. 7, the chromatography of a digest of DNA on DEAE-cellulose was remarkably similar to that of an RNA digest (Fig. 1). Mononucleotides accounted for less than 2% of the total absorbance at 260 $m\mu$, and there was no material in a position characteristic of an oligonucleotide larger than tetranucleotides. It was concluded that the *S. marcescens* nuclease acts in a similar manner to hydrolyze both RNA and DNA substrates.

Position of Terminal Phosphoryl Group—The location of the

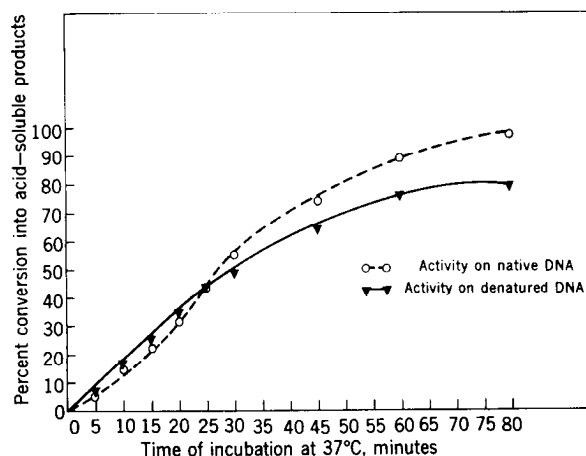


FIG. 6. Time course of hydrolysis of native and denatured DNA. The DNA solutions were prepared as described under "Materials" and prewarmed to 37°. The reactions were started by the addition of 1.5 ml of a 60-fold dilution of enzyme in standard assay buffer to 6 ml of each DNA solution. At the indicated times, 0.5-ml aliquots were removed and immediately precipitated with 0.5 ml of cold 4% perchloric acid. The assays were completed as usual. The zero-time sample was subtracted from the later readings and the results plotted as percentage of conversion to acid-soluble products.

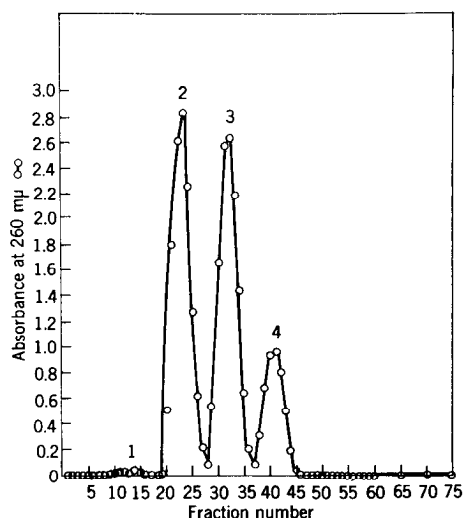


FIG. 7. DEAE-cellulose chromatography of an *S. marcescens* nuclease digest of salmon sperm DNA. The digestion mixture consisted of 10 mg of salmon sperm DNA in 2 ml of 0.1 M Tris-HCl, pH 8.2, 0.01 M MgCl₂ to which 0.1 ml of an 8-fold concentrate of the Fraction 7 enzyme (containing 38,000 units per ml) was added. After 8 hours of incubation at 37°, another 0.2 ml of the enzyme was added. After a total of 30 hours of incubation, 1.2 ml of the digestion mixture were combined with 1.2 ml of 0.02 M Tris-HCl, pH 7.5, containing 7 M urea (urea-buffer) and applied to a DEAE-cellulose column (1 × 25 cm) which had been equilibrated with the urea-buffer. The digestion mixture was washed onto the column with 2 ml of urea-buffer, and eluted with a linear gradient consisting of 1 liter of 0.0 to 0.4 M NaCl in the urea-buffer. Fractions (8 ml) were collected at the rate of 2 ml per min, and the absorbance of each fraction determined at 260 mμ.

terminal phosphate on the products of DNA digestion was determined in the same manner as that described for the RNA substrate. In agreement with those results, the products of digestion of DNA by the *S. marcescens* nuclease in conjunction with venom phosphodiesterase were all shown by chromatographic and electrophoretic analysis to be 5'-mononucleotides.

Base Specificity—The dinucleotide fraction from the DEAE-cellulose column illustrated in Fig. 7 was analyzed in a manner similar to that described for ribonucleotides except that enzymatic methods replaced alkaline hydrolysis. The fractions comprising the dinucleotides were combined and desalted as described under "Experimental Procedure." The 5'-phosphoryl group was removed by digestion with intestinal alkaline phosphatase under the conditions described in the legend to Table I. The sample was then chromatographed on untreated Whatman No. 3MM paper with the solvent system discussed under "Methods." Under these conditions, the dinucleoside monophosphates run as a single wide band between the nucleotides and nucleosides. This band was eluted with water and concentrated to dryness. The sample was reconstituted in 0.15 ml of 0.1 M Tris HCl, pH 9.0, and then treated with 0.05 ml of venom phosphodiesterase (1 mg per ml in 0.1 M Tris-HCl, pH 9.0). The digestion mixture was incubated 2 hours at 37°, and then chromatographed on previously treated paper as described under "Methods." The separated nucleotides (3' terminus) and nucleosides (5' terminus) were cut out and eluted in 2 ml of 0.01 M HCl and their spectra determined. The molar amounts of the samples were calculated from the spectral data.

The results of this experiment showed that the enzyme had little base preference at either the 3' or 5' terminus and indicated

that there is no absolute base specificity in the attack of the *S. marcescens* nuclease on DNA substrates.

DISCUSSION

The purified *S. marcescens* nuclease is able to attack both single-stranded and double-stranded DNA and RNA at nearly equivalent rates. In this respect, the enzyme differs markedly from other microbial nucleases, which usually show some dependence on the secondary structure of the substrate. Nucleases are known which preferentially attack either native DNA (*e.g.* *Escherichia coli* exonuclease III (8)) or denatured DNA (*e.g.* *E. coli* exonuclease I (9) and micrococcal nuclease (10)). Similarly, there are microbial nucleases which preferentially hydrolyze single-stranded RNA (*e.g.* *E. coli* ribonuclease II (11)) and double-stranded RNA (*E. coli* ribonuclease III (12)). Few microbial nucleases have been found, however, which can hydrolyze both single- and double-stranded nucleic acids equally well.

Under conditions of extensive degradation, digestion of RNA or DNA by the *S. marcescens* nuclease results in the production of oligonucleotide fragments of definite length; essentially only di-, tri-, and tetranucleotides terminating in 5'-phosphate are produced. When further digested by the enzyme (Fig. 3), pentanucleotides obtained from a limited digestion of RNA are degraded mainly to di- and trinucleotides. It is significant that both RNA and DNA substrates seem to be degraded to the same set of products which provides further evidence consistent with the conclusion that both nucleolytic activities are associated with the same protein.

When the *S. marcescens* enzyme is compared with other nucleases, it is found to most closely resemble two nucleases from organs of higher animals. These enzymes, isolated from chicken pancreas (13) and rat liver (14, 15), are both Mg⁺⁺- and Mn⁺⁺-activated endonucleases which cleave RNA and DNA to oligonucleotides terminating in a 5'-phosphoryl group. The enzyme from chicken pancreas is reported to produce mainly di-, tri-, and tetranucleotides. No data are available on the size of products produced by the rat liver enzyme in its present state of purification. These enzymes differ from the *S. marcescens* nuclease in that they both preferentially attack denatured DNA. Moreover, the RNase and DNase activities of the chicken pancreas nuclease differ in several respects, indicating the possibility that separate enzymes might be involved in its action.

The *S. marcescens* nuclease differs from other microbial nucleases in a number of significant properties. The enzymes of *E. coli* (16) are all sugar-specific, acting exclusively on either RNA or DNA. *E. coli* endonuclease I resembles the DNase activity of the *Serratia* enzyme in that it is an endonuclease activated by Mg⁺⁺ or Mn⁺⁺ which leaves 5'-terminated oligonucleotides. These fragments are longer, however, with an average chain length of about 7, and the *E. coli* enzyme also acts preferentially on native DNA (17).

The *S. marcescens* enzyme is also very different from other microbial phosphodiesterases which can hydrolyze both RNA and DNA. It differs from the exonuclease from *Bacillus subtilis* (18), which produces exclusively nucleoside 3'-monophosphates, and from the *Neurospora crassa* endonuclease (19) which also produces monophosphates and is preferentially active on denatured substrates. It also differs from the extracellular enzyme from *Micrococcus sodonensis* (20), an endonuclease which, in its present state of purification, leaves only nucleosides, and from micrococcal nuclease (*cf.* Reference 1).

The purified *S. marcescens* nuclease is relatively easy to prepare, and it is stable for 6 months or longer when stored at refrigerator temperatures. It is able to degrade native and denatured DNA and RNA substrates completely to di-, tri-, and tetranucleotides, and is thus potentially useful for studies of nucleic acid sequence, particularly with oligonucleotides of moderate chain length. When used in conjunction with venom phosphodiesterase, it also provides a convenient enzymatic reagent for the complete degradation of RNA and DNA to nucleoside 5'-monophosphates.

Because of its high specific activity, it may be used in preference to the DNase I-venom phosphodiesterase combination for degrading DNA to 5'-mononucleotides, or to combinations of venom phosphodiesterase with the *Neurospora* enzyme (for degrading RNA and DNA) or RNase II (for degrading RNA) which are restricted to denatured substrates. The *Serratia* and venom enzymes may thus be the method of choice for degrading double-stranded RNA to 5'-mononucleotides. These enzymes may also be used as a system complementary to alkaline hydrolysis of RNA or to the micrococcal nuclease-spleen phosphodiesterase combination which degrades RNA and DNA to 3'-mononucleotides. The *S. marcescens* nuclease is, therefore, potentially valuable as a reagent for the study of polynucleotide structure and composition.

REFERENCES

1. NESTLE, M., AND ROBERTS, W. K., *J. Biol. Chem.*, **244**, 5213 (1969).
2. ROBERTS, W. K., NEWMAN, J. F. E., AND RUECKERT, R. R., *J. Mol. Biol.*, **15**, 92 (1966).
3. PETERSON, E. A., AND SOBER, H. P., in S. P. COLOWICK AND N. O. KAPLAN (Editors), *Methods in enzymology*, Vol. V, Academic Press, New York, 1962, p. 3.
4. TOMLINSON, R. V., AND TENER, G. M., *J. Amer. Chem. Soc.*, **84**, 2644 (1962).
5. RUSHIZKY, G. W., AND SOBER, H. A., *Biochim. Biophys. Acta*, **55**, 217 (1962).
6. NESTLE, M., AND ROBERTS, W. K., *Anal. Biochem.*, **22**, 349 (1968).
7. RAZZELL, W. D., AND KHORANA, H. G., *J. Biol. Chem.*, **234**, 2114 (1959).
8. RICHARDSON, C. C., LEHMAN, I. R., AND KORNBERG, A., *J. Biol. Chem.*, **239**, 251 (1964).
9. LEHMAN, I. R., AND NUSSBAUM, A. L., *J. Biol. Chem.*, **239**, 2628 (1964).
10. DIRKSON, M. L., AND DEKKER, C. A., *Biochem. Biophys. Res. Commun.*, **2**, 147 (1960).
11. SINGER, M. F., AND TOLBERT, G., *Biochemistry*, **4**, 1319 (1965).
12. ROBERTSON, H. D., WEBSTER, R. E., AND ZINDER, N. D., *J. Biol. Chem.*, **243**, 82 (1968).
13. ELEY, J., AND ROTH, J. S., *J. Biol. Chem.*, **241**, 3063, 3070 (1966).
14. CURTIS, P. J., BURDON, M. G., AND SMELLIE, R. M. S., *Biochem. J.*, **98**, 813 (1966).
15. CURTIS, P. J., AND SMELLIE, R. M. S., *Biochem. J.*, **98**, 818 (1966).
16. LEHMAN, I. R., *Progr. Nucl. Acid Res.*, **2**, 83 (1963).
17. LEHMAN, I. R., ROUSSOS, G. G., AND PRATT, E. A., *J. Biol. Chem.*, **237**, 819 (1962).
18. KERR, I. M., CHIEN, J. R., AND LEHMAN, I. R., *J. Biol. Chem.*, **242**, 2700 (1967).
19. LINN, S., AND LEHMAN, I. R., *J. Biol. Chem.*, **240**, 1287, 1294 (1965).
20. BERRY, S. A., AND CAMPBELL, J. N., *Biochim Biophys. Acta*, **132**, 78, 84 (1967).

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