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THE NUCLEASES OF YEAST

II. PURIFICATION, PROPERTIES AND SPECIFICITY OF AN ENDONUCLEASE FROM YEAST

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SUMMARY

A nuclease has been purified approx. 30-fold from the supernatant fraction of a hybrid yeast *(Saccharomyces fragilis* × *Saccharomyces dobzhanskii)* by salt fractionation, chromatography on Sephadex G-200, and DEAE-cellulose chromatography.

The optimum pH is 7.6 and Mg^{2+} is required for the full activity.

The action of this nuclease on polyribonucleotides is exclusively endonucleolytic. The major products upon extensive digestion of homopolymers are di- and trinucleotides having 5′-phosphomonoester end groups. The formation of mononucleotide is slight. Polyadenylic acid and polyuridylic acid are hydrolyzed first to a family of small oligonucleotides having 5′-phosphomonoester end groups. The distribution of these oligonucleotides is not completely random and depends on the conformation of the substrate. Hydrolysis of shorter chains is much slower than longer chains. This endonuclease has no apparent specificity for a particular base residue in polynucleotides. This mode of hydrolysis coupled with its high stability make this enzyme a very useful reagent for the preparation of oligonucleotides with 5′-phosphate ends from either synthetic polynucleotides or natural RNA.

This enzyme appears to be specific for polynucleotides having a random coil conformation. Double- and triple-stranded helical conformations are less susceptible to attack. Polyadenylic acid, polyuridylic acid and polyinosinic acid are hydrolyzed much faster than polycytidylic acid, transfer RNA or ribosomal RNA.

The enzyme preparation hydrolyzes denatured DNA at approximately the same rate as yeast ribosomal RNA. Highly polymerized native *Escherichia coli* DNA is almost inactive as substrate.

Abbreviations and conventions: poly (A), polyadenylic acid; poly (U), polyuridylic acid; poly (C), polycytidylic acid; poly (I), polyinosinic acid; tRNA, transfer RNA; rRNA, ribosomal RNA. One $A_{260\ m\mu}$ unit is the amount of nucleotide which gives 1 ml of solution of unit absorbance at pH 7, 260 mμ in a 1-cm light path.

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INTRODUCTION

The preceding paper described the existence of several RNA-degrading enzymes in yeast and the purification and some of the properties of a nuclease in the supernatant fraction[1]. Preliminary studies implied that this nuclease was an endonuclease with specificity of practical use in preparatory chemistry of oligonucleotides as well as for structure studies of nucleic acid. The principal products after extensive digestion were shown to be dinucleotides and trinucleotides having 5'-phosphate termini. The present studies are concerned with details of the base and conformation specificity and possible applications of this enzyme.

No differences in specificity were detected between nuclease I and II in the preceding paper[1]. In the experiments to be described, nuclease II was used throughout. For more definitive results, highly purified and well characterized substrates were used. Kinetics of hydrolysis of the substrate were followed by the formation of new end groups instead of by acid-solubilization of substrate.

MATERIALS AND METHODS

Substrates

Yeast transfer RNA (tRNA) and ribosomal RNA (rRNA) were prepared from log-phase *Saccharomyces lactis*, grown in enriched medium according to the methods described by CHERAYIL AND BOCK[2] and BRUENING[3], respectively. *Escherichia coli* K12 DNA (Lot No. 640661) was purchased from General Biochemicals. Polyadenylic acid (poly (A)), polyuridylic acid (poly (U)), polycytidylic acid (poly (C)) and polyinosinic acid (poly (I)) were obtained from Miles Chemical Co. All the random copolymers, poly (A,G), poly (C, U), poly (I, U), poly (I, C) and poly (I, A) were synthesized from corresponding mononucleotide diphosphates (products of P–L Biochemicals, Inc.) using polynucleotide phosphorylase purified from *Micrococcus lysodeikticus*. The purification procedure was a modification of the procedures of STEINER AND BEERS[4] and of SINGER AND O'BRIEN[5]. All the synthetic polynucleotides were chromatographed on Sephadex G-50 and lyophilized.

Enzyme isolation

The concentrated DEAE fraction corresponding to nuclease II in the preceding paper[1] was used in all experiments to be described. The enzyme was purified in the same manner as previously described except: (1) crude extract was treated with streptomycin sulfate (final concn. 0.4%); (2) the enzyme fraction from DEAE chromatography was concentrated by dialyzing against saturated ammonium sulfate and the precipitate was collected directly without addition of bovine plasma albumin. The specific activity of this yeast endonuclease preparation was 702 units/mg. The preparation was found to have negligible non-specific phosphodiesterase activity as assayed using bis(*p*-nitrophenyl) phosphate as substrate, and negligible phosphomonoesterase activity as assayed with *p*-nitrophenyl phosphate, 3'-AMP and 5'-AMP as substrate. The enzyme solution was stored in 0.02 M Tris–HCl, pH 7.6.

Although not employed for this preparation, in later preparations it was found that chromatography on a hydroxylapatite column was very effective for the further purification of this enzyme. The enzyme is eluted around 0.3 M K_2HPO_4 buffer at

pH 7.6 during gradient elution from hydroxylapatite. Absorption in a small hydroxylapatite column is also very effective for concentrating diluted enzyme solutions. These steps are now used routinely in this laboratory.

Enzyme assay

The assay of the yeast endonuclease with yeast RNA was performed as described in the preceding paper[1] except that a buffer containing 0.2 M Tris–HCl (pH 7.6) and 0.02 M $MgCl_2$ was used instead of 0.5 M Tris–HCl (pH 8.0). A unit of enzyme is defined as the amount of enzyme which, under the above conditions, gives absorbance of 0.01 at 260 mμ. This assay is linear up to 0.6 absorbance. Specific activity is defined as units/mg of protein.

Base composition of substrates

Two $A_{260\ m\mu}$ units were hydrolyzed in 0.1 M KOH at 100° for 20 min and mononucleotides in the hydrolysate were separated on a Dowex 1-X8 microcolumn (1.5 mm i.d. × 5 mm length) by a procedure similar to that described by NAKAO *et al.*[6] The base ratio was calculated from the spectra and the molar absorbances of the corresponding nucleotides.

Chromatography and characterization of yeast endonuclease digests

A. DEAE-cellulose chromatography of yeast endonuclease digests. For the separation on the basis of chain length of the oligonucleotides resulting from the digestion of polyribonucleotides, DEAE-cellulose column chromatography was carried out with a procedure similar to that described by TOMLINSON AND TENER[7]. Unless otherwise stated, the effluent from the columns was monitored by passing it through a flow cell of 5 mm path length in a Beckman DB spectrophotometer and recording the output with a Varian G-14 recorder.

At the end of the incubation, the digest was deproteinized by extracting with sodium dodecyl sulfate–phenol and was loaded on a DEAE-cellulose column. Elution conditions were varied slightly depending upon the sample under study. The specific conditions can be found in the legends to the figures.

B. Characterization of the products. The peaks from the DEAE-cellulose columns were characterized by alkaline hydrolysis and chromatography on Whatman No. 1 paper in a descending isobutyric acid–conc. NH_4OH–water (66:1:33, v/v/v) system[8]. The products were identified by comparing their R_F values to those of reference compounds. For quantitative measurements, the spots were eluted and ultraviolet spectra of each solution were taken.

The chain length of oligonucleotides resulting from the digest of homopolymers was conveniently determined by measuring the ratio of the nucleoside to the sum of nucleoside *plus* nucleotides in the alkaline hydrolysate of an oligonucleotide.

C. Analysis of the terminal residues in the yeast endonuclease digestion products. The nucleoside fraction was separated from the alkaline hydrolysate of the nuclease digest by a microcolumn of DEAE-cellulose (0.5 cm^2 × 1 cm) and was chromatographed on Whatman No. 1 using the descending system of LANE[9]. Identification and quantification performed as in B.

Kinetics of hydrolysis by yeast endonuclease

For more satisfactory assays for kinetic studies, the rate of hydrolysis of the substrates was measured on the basis of the formation of new end groups. The formation of new end groups was quantitatively determined by measuring the amount of nucleoside or nucleoside diphosphate in the alkaline hydrolysate of a digest at a given incubation time, using procedures described above.

RESULTS

A. Rates of hydrolysis of synthetic polymers and RNA

Since non-linearity between acid-soluble products formed and enzyme activity was often found with synthetic polymers or long-chain RNA as substrates, the rate of hydrolysis was measured on the basis of the formation of new end groups, as described under METHODS. The results are summarized in Table I.

TABLE I

NUCLEOSIDE AND NUCLEOSIDE 3'(2'),5'-DIPHOSPHATE YIELD AFTER ALKALINE HYDROLYSIS OF ENZYMIC DIGESTS OF VARIOUS SUBSTRATES

2 μmoles (organic phosphate) of each substrate was incubated with 30 units of yeast endonuclease in a reaction mixture of 0.5 ml containing 10^{-3} M $MgCl_2$ and 0.1 M Tris–HCl, pH 7.6, at 37°. 0.1-ml aliquots of the reaction mixture were removed at the times indicated and hydrolyzed in 0.1 M KOH for 20 min at 100°. The resulting hydrolysates were then analyzed for nucleoside and nucleoside 3'(2'),5'-diphosphate.

	Nucleoside (μmoles)				*Nucleoside 3'(2')5'-diphosphate (μmoles)*			
	0 min	*30 min*	*60 min*	*120 min*	*0 min*	*30 min*	*60 min*	*120 min*
Poly (A)	0.010	0.036	0.064	0.109	0.0082	0.031	0.062	0.102
Poly (U)	0.010	0.045	0.069	0.098	0.0096	0.042	0.065	0.099
Poly (C)	0.011	0.015	0.022	0.028	0.0088	0.013	0.021	0.026
Poly (I)	0.010	0.059	0.117	0.142	0.011	0.060	0.108	0.138
rRNA	0.0072	0.015	0.023	0.054	0.0061	0.012	0.022	0.052
tRNA	0.0084	0.014	0.023	0.046	0.0081	0.012	0.020	0.047

The diester bond cleavage in a given incubation time was calculated by taking the average of nucleoside and nucleoside 3'(2'),5'-diphosphate produced in the alkaline hydrolysate and subtracting the zero time value from the average. The results are plotted in Fig. 1. The diester bonds cleaved are expressed as μmoles per total incubation mixture.

B. Products of yeast endonuclease digests

2 mg of each of the synthetic homopolymers poly (A), poly (U) and poly (I) were digested with the enzyme for prolonged periods. After extracting the enzyme protein with sodium dodecyl sulfate–phenol, the digests were chromatographed on a DEAE-cellulose column with a linear gradient of ammonium acetate at pH 7.6, and the peaks were recovered and characterized, as described in detail in METHODS.

All the digests yielded three major peaks but the first peak, which was identified as nucleoside 5'-phosphate, was always quantitatively less significant in the digests.

TABLE II

PRODUCTS OF SYNTHETIC HOMOPOLYMERS AFTER PROLONGED DIGESTIONS

Polymers	*Enzyme added (units)*	*$MgCl_2$ final concn. (M)*	*Incubation time (h)*	*Products**		
				1st peak	*2nd peak*	*3rd peak*
Poly (A)	10	$5 \cdot 10^{-3}$	8	pA (3%)	pApA (49%)	pApApA (48%)
Poly (U)	26	10^{-2}	20	pU (8%)	pUpU (58%)	pUpUpU (34%)
Poly (I)	20	$2.5 \cdot 10^{-3}$	8	pI (17%)	pIpI (48%)	pIpIpI (35%)

* The composition of products is calculated from the % of ultraviolet absorbance eluted in each peak.

The digestion conditions and the compositions of the products are summarized in Table II.

From the results obtained, it can be concluded that the major products upon an extensive digestion are mononucleotide, dinucleotide and trinucleotide with 5′-phosphorylated ends and that the dinucleotide and the trinucleotide markedly resist further degradation. It will be shown that the yields of mononucleotide during limited digestion are insignificant.

C. Specificity for nucleoside residues

As depicted in Fig. 1, the reaction rates depend largely upon the substrates used. In order to find out whether there is any specificity for or against diester bonds

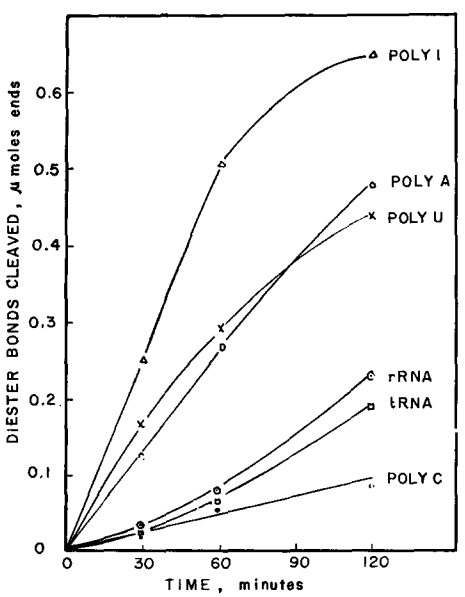

Fig. 1. Rates of hydrolysis of synthetic polymers and RNA. The data in Table I is plotted in Fig. 1. The phosphodiester bonds cleaved, expressed as μmoles per total incubation mixture, are calculated by taking the average of nucleoside and nucleoside 3′(2′),5′-diphosphate produced in the alkaline hydrolysis and subtracting the zero time values from the average.

TABLE III

A. COMPOSITION OF 3′-TERMINAL BASES IN THE YEAST ENDONUCLEASE PRODUCTS

In 0.7 ml of reaction mixture contained 0.1 M Tris–HCl (pH 7.6), 0.5 mM $MgCl_2$ for poly (A, G) and poly (C, U), 0.01 M $MgCl_2$ for yeast rRNA, substrate and enzyme as indicated. The reaction mixtures were incubated at 37° for 8 h and then analyzed for identity of the 3′-terminal base.

Polymer	*Amount* *μmoles P*	*Enzyme units*	*Nucleoside after alkaline hydrolysis (μmoles)*			
			A	*G*	*C*	*U*
Poly (A, G)	1.0	49	0.156	0.112	—	—
Poly (C, U)	1.5	74	—	—	0.30	0.312
Yeast rRNA	4.0	98	0.086	0.083	0.064	0.092

B. PERCENT COMPOSITION OF 3′-TERMINAL BASES IN YEAST ENDONUCLEASE PRODUCTS

Figures in parentheses indicate % base composition in the substrate polymer.

Polymer	*% Composition*			
	A	*G*	*C*	*U*
Poly (A, G)	58.2 (60.0)	41.8 (40.0)	—	—
Poly (C, U)	—	—	49.0 (47.6)	51.0 (52.4)
Yeast rRNA	26.4 (27.6)	25.6 (26.2)	19.7 (19.8)	28.3 (26.4)

involving certain nucleoside residues, enzymically synthesized co-polymers and RNA were digested with the enzyme and the terminal residues in the products were analyzed to compare with the base ratios in the substrates.

Poly (A, G), poly (C, U), and yeast rRNA were digested with the nuclease under the condition given in Table III. After the enzyme protein was removed by sodium dodecyl sulfate–phenol extraction, the digests were then hydrolyzed in 0.1 M NaOH for 20 h at 37° and analyzed for nucleoside content .

The composition of terminal residues in the products are shown in Table IIIA. The percent composition of the terminal residues in the products are also compared with the base ratios of the substrates in Table IIIB.

As seen in Table IIIB, the composition of the terminal residues in the yeast endonuclease products are very close to the base ratios of the substrates. This indicates, therefore, that the enzyme is non-specific for the nucleoside residues within the polynucleotide chain, and the difference in the rate of degradation of substrates by this enzyme is due largely to factors other than base specificity, possibly to conformation of substrates.

D. Effect of substrate conformation on the rate of degradation

The rates of hydrolysis of the polymers with different secondary structure were determined by the acid-solubilization assay. The extents of hydrolysis were from 1% up to 45% of the substrates made acid-soluble. DNA was denatured by heating at 100° for 10 min in 0.02 M Tris–HCl (pH 7.6), at a concentration of 1 μmole nucleotide per ml and then cooled rapidly by immersion in ice. The assay conditions and the results obtained are given in Table IV. It was assumed that the base

TABLE IV

EFFECT OF SUBSTRATE CONFORMATION ON RATE OF HYDROLYSIS

Reaction mixture (0.3 ml) contained 200 nmoles of substrate, 30 μmoles of Tris–HCl (pH 7.6), 0.3 μmoles of $MgCl_2$, and 3.5 units of yeast endonuclease. After incubating at 37° for 30 min or 60 min as indicated, 0.2 ml of carrier (2 mg/ml yeast rRNA) and 0.5 ml of 0.025% uranyl acetate in 6% $HClO_4$ were added. The mixture was kept in ice for 10 min, and then the precipitate was removed by centrifugation. Absorbance of the supernatant was read at 260 mμ against a blank containing no enzyme.

Substrates	$A_{260\ m\mu}$	*Acid-soluble nucleotide formed nmoles/30 min*
Poly (A)	1.03	71
Poly (U)	0.91	91
Poly (A) + poly (U)	0.16	13.1
Poly (A) + 2 poly (U)	0.20	17.4
rRNA	0.22	20.2
		nmoles/60 min
E. coli DNA	0.04	4
Heated *E. coli* DNA	0.52	52
rRNA	0.44	44

ratios of the acid-soluble fragments produced from the substrates were the same as those of substrates and the molar absorbances at 260 mμ for the acid-soluble fragments were equal to those of their constituent mononucleotides at acid pH. STEVENS AND FELSENFELD[10] reported that a 1:1 mixture of poly (A) and poly (U) gives a two-strand helical complex of poly (A) and poly (U), and that a 1:2 mixture of poly (A) and poly (U) gives three-strand helical complex under the incubation conditions used here. From the data obtained, it appears that the rate at which substrates are attacked is related to the extent of ordered structure of polymers. The polymers having helical structure are more resistant to the nuclease attack than those having single-stranded random coil structure.

E. The course of degradation and the products of partial digestion

The partial digests of homopolymers at the different enzyme levels and incubation periods were chromatographed on a DEAE-cellulose column using a NaCl gradient and products were identified as described in METHODS.

The partial digestion of poly (A) produced a continuous series of $(pA)_n$ homologs starting with dinucleotides. Upon further digestion with more enzyme or more prolonged incubation, oligonucleotides accumulated with nearly the same relative abundance without change from the general pattern of distribution of oligonucleotides shown in Fig. 2 until all the unresolved long chain peak disappeared. The pA peak did not appear in appreciable amount until all oligonucleotides longer than trinucleotides had disappeared. Therefore poly (A) can be digested to pApA and pApApA with only a limited amount of pA produced. The resistance of ordered regions of the substrate to the enzymic attack may be responsible for this non-random distribution.

Like poly (A), the partial digestion of poly U produced a continuous series of

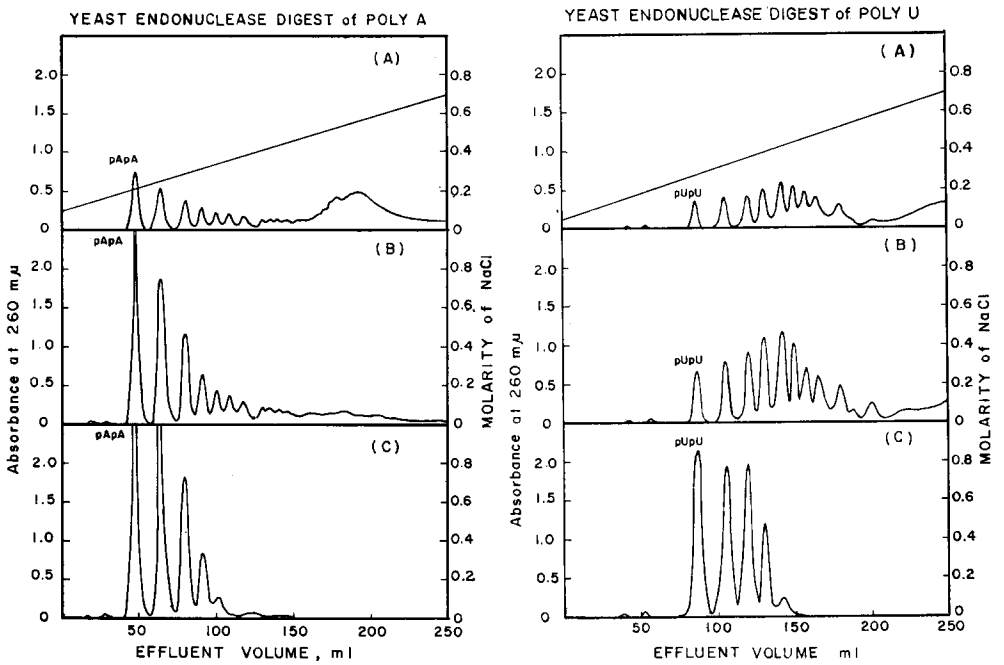

Fig. 2. DEAE-cellulose chromatography of yeast endonuclease digests of poly (A). 2-mg portions of poly (A) in 1.0 ml of 0.1 M Tris–HCl (pH 7.6) were digested at 37° with (A) 13 units of the enzyme for 2 h, (B) 26 units of the enzyme for 2 h, or (C) 26 units of the enzyme for 6 h. After extracting enzyme protein with sodium dodecyl sulfate–phenol, the digests were chromatographed on a 0.5 $cm^2 \times 30$ cm column of DEAE-cellulose with a linear gradient of NaCl from 0.1 M to 0.7 M in a total volume of 250 ml buffer containing 0.02 M Tris–HCl, pH 7.6, and 7 M urea. The flow rate was held constant at 10 ml/h. The straight line indicates the molarity of NaCl in the buffer entering the column. The first four peaks were identified as pApA, pApApA, pApApApA and pApApApApA, respectively, as described in METHODS.

Fig. 3. DEAE-cellulose chromatography of yeast endonuclease digests of poly (U). 2-mg portions of poly (U) in 0.8 ml of 0.08 M Tris–HCl (pH 7.6) were digested at 37° with (A) 10.4 units of the enzyme for 2 h, (B) 20.8 units of the enzyme for 2 h, or (C) 41.6 units of the enzyme for 6 h. After removing the enzyme protein with sodium dodecyl sulfate–phenol extraction, the digests were chromatographed on a 0.5 $cm^2 \times 30$ cm column of DEAE-cellulose with a linear gradient of NaCl from 0.05 M to 0.7 M in a total volume of 250 ml of 0.02 M Tris–HCl (pH 7.6). The flow rate was held constant at 10 ml/h. The straight line indicates the molarity of NaCl in the buffer entering the column. The first four peaks were identified as pUpU, pUpUpU, pUpUpUpU and pUpUpUpUpU, respectively, as described in METHODS.

$(pU)_n$ homologs starting with pUpU. The course of degradation was similar to poly (A) digestion except that the distribution pattern of short chains of oligomers was as shown in Fig. 3. $(pU)_6$ was always the biggest peak among the oligomers when an appreciable amount of undegraded polymer was left. Further digestion led to accumulation of the short chains of oligonucleotides with nearly the same distribution until the unresolved peak of long-chain substrate had almost disappeared. The maximum peak subsequently shifted to a shorter chain length. A small pU peak appeared when the $(pU)_5$ peak had almost disappeared. The accumulation of oligonucleotides indicates resistance of the short chains to enzymic attack. Their distribution was random even though the overall hydrolysis of poly (U) was not.

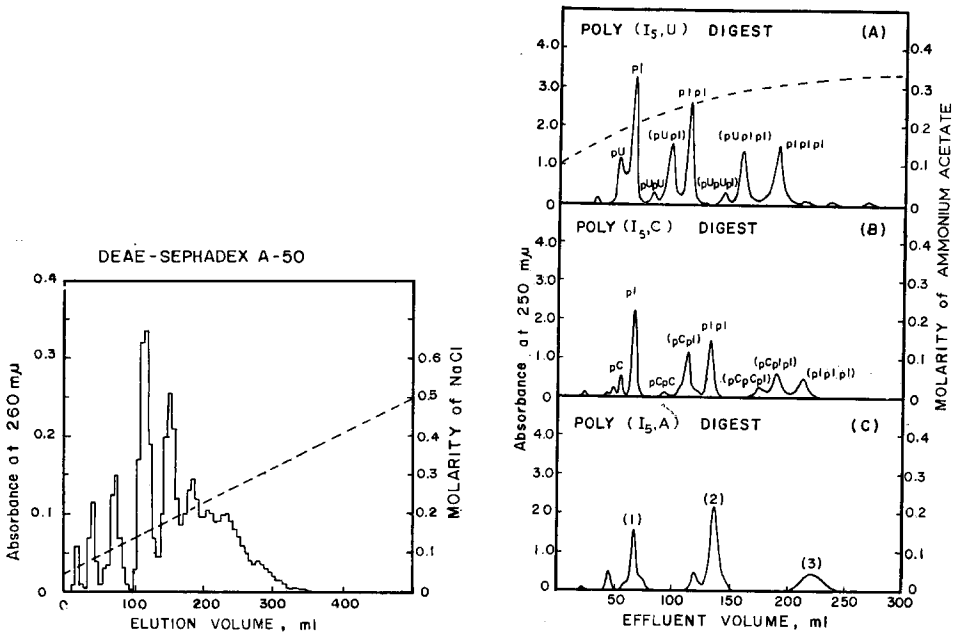

Fig. 4. DEAE-Sephadex A-50 chromatography of yeast endonuclease digests of yeast RNA. The reaction mixture contained 1 mg of purified commercial yeast RNA and 12.5 units of yeast endonuclease in 0.4 ml of a buffer (pH 7.6), 0.1 M in Tris–HCl, and 0.01 M in $MgCl_2$. The reaction mixture was incubated at 37° for 5 h, and then chromatographed on a 0.5 cm^2 × 30 cm column of DEAE-Sephadex A-50 with a linear gradient of NaCl from 0.05–0.5 M in a total volume of 500 ml buffer containing 0.02 M Tris–HCl (pH 7.6) and 7 M urea. Fractions of 5.5 ml were collected using a flow rate of 11 ml/h. The identification of products was not attempted.

Fig. 5. DEAE-cellulose chromatography of yeast endonuclease digests of random co-polymers poly(I, U) (5:1), poly (I, C) (5:1) and poly (I, A) (5:1). 5 mg of polymer, in 1.0 ml solution of 0.1 M Tris–HCl (pH 7.6), 2.5 mM in $MgCl_2$ and 10 mM in Na_2HPO_4, was incubated with 52 units of yeast endonuclease at 37° for 9 h. After incubation, the enzyme protein was extracted with sodium dodecyl sulfate–phenol and then the digest was chromatographed on a 0.5 cm^2 × 30 cm column of DEAE-cellulose with an exponential gradient from 0.1–0.36 M ammonium acetate (pH 7.6) with 125 ml of 0.1 M ammonium acetate (pH 7.6) in the constant volume mixing flask. The flow rate was kept constant at 10 ml/h. The dotted line indicates the molarity of ammonium acetate entering the column. Peaks were tentatively identified by judging from the chromatographic behavior and ultraviolet spectra. Rechromatography of peak (3) in poly (I, A) (5:1) digest on a DEAE-cellulose column at pH 4.0 gave four peaks, but further identification was not attempted. The parentheses on the figure designate mixtures of sequence isomers.

A chromatogram of partially digested yeast RNA is shown in Fig. 4. Characterization of products was not attempted, but the oligonucleotide distribution showed no unusual features if compared to poly (U) and poly (A) digests.

F. Some applications of yeast nuclease to the preparation of specific oligonucleotides

Since short-chain oligonucleotides were relatively resistant to this enzyme, homo-oligonucleotides bearing 5′-monophosphate termini could be obtained in a good yield from their appropriate homopolymers. The enzyme was also applicable for the preparation of oligonucleotides consisting of more than two bases, but this application is limited by the chromatographic resolution of products. Fig. 5 shows

an example of such application. Synthetic random co-polymers, poly (I, U) (5:1)*, poly (I, C) (5:1)*, and poly (I, A) (5:1)* were subjected to a prolonged digestion by the enzyme, and the digests were chromatographed on a DEAE-cellulose column.

DISCUSSION

Nuclease II from yeast *(Saccharomyces fragilis* × *Saccharomyces dobzhanskii)* is an endonuclease. The absence of mononucleotide formation during the early stages of the hydrolysis and the accumulation of di- and trinucleotides with only limited formation of mononucleotide after prolonged hydrolysis suggest that the phosphodiester bonds adjacent to either end of oligonucleotides are extremely resistant to cleavage. It has been shown that the enzyme described here had no apparent specificity for the nucleoside adjacent to either side of the phosphodiester bond attacked. The rate of hydrolysis appeared to be most influenced by the secondary structure of the substrate.

The rate of hydrolysis of poly (C) is unexpectedly lower and that of poly (I) is greater than the rate of hydrolysis of poly (U), which is known to be a random coil with no secondary structure. This may be the result of the complex effect of pH, temperature, and ionic strength and composition on the polynucleotide conformation in solution; however, the explanation of this result is not readily apparent from the data available.

Although the enzyme preparation used in this work has been purified only 30-fold over the original cell extract (the actual purification might be higher since this value was based on the total activity of all RNA degrading enzymes), analysis of products showed that this preparation was good enough for the preparation of oligonucleotides bearing 5'-phosphomonoester ends without significant contamination of other types of oligonucleotides. The enzyme preparation contained no non-specific phosphodiesterase activity and contained only a trace of alkaline phosphatase activity which could under certain conditions be completely inhibited. We found in later preparations that rechromatography on a hydroxylapatite column was very effective for the further purification of this nuclease.

The enzyme attacks denatured DNA and yeast rRNA at approximately the same rate. The conditions which were optimal for RNA were generally those giving maximum rates for hydrolysis of denatured DNA. The two activities co-chromatographed on both Sephadex G-200 and DEAE-cellulose columns. Thus it appears that the same enzyme catalyzes both types of hydrolytic reactions. The highly polymerized native *E. coli* DNA was almost inert as substrate. This requirement for non-ordered polynucleotide substrates is additional support that the same enzyme is involved in the hydrolysis of both RNA and DNA. Nevertheless, the possibility that deoxyribonuclease activity is a contaminant rather than that a single enzyme is responsible for both activities, is not completely eliminated at this stage of purification.

The specificity of yeast endonuclease shows that this enzyme is entirely different from the other yeast nucleases reported by OHTAKA, UCHIDA AND SAKAI[11] and by DANNER AND MORGAN[12] since in both cases these nucleases hydrolyze RNA into

* The input ratio of nucleoside diphosphates during synthesis by polynucleotide phosphorylase.

nucleoside 3′-monophosphates. The mode of hydrolysis by yeast endonuclease and its other properties are, however, very similar to those of the nucleases from liver nuclei[13], *Azotobacter agilis*[14], and *Neurospora crassa*[15]. NAKAO, NOGAMI AND OGATA[16] have obtained tentative evidence that a nuclease which cleaves both DNA and RNA into 5′-phosphate terminated oligonucleotides also occurs in *Rhodotorula glutinis*. It is of interest that such similar nucleases are widespread among organisms although the products of such nucleases seem useless for replenishing the pool of nucleoside 5′-phosphates. The physiological role of these enzymes is uncertain.

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REFERENCES

1 Y. NAKAO, S. Y. LEE, H. O. HALVORSON AND R. M. BOCK, *Biochim. Biophys. Acta*, 151 (1968) 114.
2 J. D. CHERAYIL AND R. M. BOCK, *Biochemistry*, 4 (1965) 1174.
3 G. E. BRUENING, *Ph. D. Thesis*, University of Wisconsin, Madison, Wisc., 1965.
4 R. F. STEINER AND R. F. BEERS, *Polynucleotides*, Elsevier, New York, 1961.
5 M. F. SINGER AND B. M. O'BRIEN, *J. Biol. Chem.*, 238 (1963) 328.
6 Y. NAKAO, A. IMADA, T. WADA AND K. OGATA, *Agr. Biol. Chem.*, 28 (1964) 151.
7 R. V. TOMLINSON AND G. M. TENER, *J. Am. Chem. Soc.*, 84 (1962) 2644.
8 Pabst Laboratories Circular OR-10, 1956.
9 B. LANE, *Biochim. Biophys. Acta*, 72 (1963) 110.
10 C. L. STEVENS AND G. FELSENFELD, *Biopolymers*, 2 (1964) 293.
11 Y. OHTAKA, K. UCHIDA AND T. SAKAI, *J. Biochem.*, 54 (1963) 322.
12 J. DANNER AND R. S. MORGAN, *Biochim. Biophys. Acta*, 76 (1963) 655.
13 L. A. HEPPEL, P. J. ORTIZ AND S. OCHOA, *Science*, 123 (1956) 415.
14 A. STEVENS AND R. J. HILMOE, *J. Biol. Chem.*, 253 (1960) 3016.
15 S. LINN AND I. R. LEHMAN, *J. Biol. Chem.*, 240 (1965) 1287, 1294.
16 Y. NAKAO, I. NOGAMI AND K. OGATA, *Agr. Biol. Chem.*, 27 (1963) 507.