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Characterization of a Yeast Endonuclease*

Ramon Piñon

ABSTRACT: A deoxyribonuclease from *Saccharomyces cerevisiae* has been purified approximately 300-fold. The enzyme has a requirement for Mg^{2+} or Mn^{2+}, which cannot be replaced by Ca^{2+}.

Its mode of action is primarily endonucleolytic, yielding oligonucleotides ending in 5′-phosphates, and it exhibits a 750-fold preference for single-stranded DNA over native DNA.

Recent evidence (Lehman, 1967; Takagi *et al.*, 1968; Grossman *et al.*, 1968) that various kinds of deoxyribonucleases are involved in the replication, repair, and recombination of genetic material make a study of the number and kind of DNase specificities in an organism such as yeast of great interest. Yeast has several characteristics which make it well suited to the molecular investigation of genetic recombination: (1) it is unicellular and has a stable diplophase in which meiosis can be induced easily and with high efficiency. Meiotic recombination thus occurs only during a restricted period during the life cycle; (2) a relatively low rate of recombination also occurs in mitotic cells and this rate can be increased greatly by ultraviolet irradiation, X-rays, or chemical mutagens (Roman and Jacob, 1958; Manney and Mortimer, 1964; Zimmerman and Schwaier, 1967). Furthermore, mitotic recombination is most readily induced at a certain stage in the division cycle, *i.e.*, just prior to the S period (Esposito, 1967); (3) the mating-type alleles a and α, influence the frequency of recombination, in that aα diploids undergo recombination, but aa or $\alpha\alpha$ diploids do not or do so rarely (Friis and Roman, 1968); (4) the genetics of yeast has been extensively studied in the past few years, and data have accumulated on various aspects of recombination (Mortimer and Hawthorne, 1969). Such data may be particularly useful in studies which attempt to relate enzyme activity with recombination. In contrast to studies with *Escherichia coli*, where extensive descriptions of purified DNases have been reported, no distinct DNases have been described in yeast. The purpose of this communication is to describe the partial purification and characterization of a DNase (endonuclease A) from *Saccharomyces cerevisiae* which exhibits a decided preference for single-stranded DNA.

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Materials and Methods

Yeast Strains. The haploid strain C252 (2d) from the yeast stocks of Dr. H. Roman, and the diploid 9-D1 were used in the work reported here. 9-D1 was the result of a cross between C252 (2d) and X173 obtained from Dr. R. K. Mortimer.

Media. Three different types of media were used for growing the two strains, and were tested with regard to the yield of DNase. Supplemented YEP medium (YEP + Ad) contained as follows (grams per liter): yeast extract, 10; peptone, 20; glucose, 20; and adenine, 0.1. Wickerham's synthetic complete medium (SC) contained: yeast nitrogen base (without amino acids), 6.7; glucose, 20; adenine, 0.01; uracil, 0.01; arginine, 0.01; and tryptophan, 0.01. Supplemented minimal medium (MA) contained: yeast nitrogen base, 6.7; glucose, 20; and adenine, 0.01.

Chemicals. Alkaline phosphatase from *E. coli* was purchased from Miles Research Laboratories. Venom phosphodiesterase and bovine spleen phosphodiesterase were purchased from Worthington Biochemical Corp. Sodium deoxycholate was obtained from Mann Research Laboratories. Carboxymethylcellulose (CM-52) was a Whatman product. Highly polymerized calf thymus DNA and phenylmethylsulfonyl fluoride were purchased from Sigma Chemical Co., and 2-mercaptoethylamine-HCl were obtained from Calbiochem. The detergent Brij-58 was a gift from McKesson Chemical Co. Glusulase, a trade name for snail digestive juice, was purchased from Endo Laboratories. Yeast extract, peptone, and yeast nitrogen base were purchased from Difco Laboratories.

^{3}H-Labeled T5 DNA. ^{3}H-Labeled T5 DNA was prepared with the cooperation of Dr. M. Feiss by the method outlined by Thomas and Abelson (1966). *E. coli* B was grown in minimal medium supplemented with 0.2% Casamino Acids and 0.25% glucose. $CaCl_2$ was added to 2×10^{-3} M at the time of phage infection. Phage T5 was added when the bacterial titer was 1×10^8/ml at a multiplicity of infection

of 0.1 phage/cell. The T5 DNA was labeled by adding [6-^{3}H]-uracil to 0.125 μCi/ml at 15-min intervals during the last hour of the 3-hr lysis period. The phage lysate was centrifuged at 5000 rpm for 10 min in the Sorvall refrigerated centrifuge. The supernatant was then centrifuged for 60 min at 19,000 rpm at 5° in the 19 rotor of the Spinco L2-65 ultracentrifuge. A small amount of TM buffer[1] was added to the phage pellet, and the resuspension was allowed to take place at 5° overnight. Two milliliters of the phage suspension was layered on a 3-ml CsCl gradient (ρ 1.3–1.6) in TM buffer, and centrifuged for 30 min at 33,000 rpm in the Spinco SW50L rotor. The phage were removed with a Pasteur pipet and dialyzed overnight against 0.01 M potassium phosphate (pH 7.0). The DNA was extracted by rolling gently at room temperature with an equal volume of distilled phenol saturated with 0.01 M potassium phosphate (pH 7.0). The phenol extraction was repeated four times. The specific absorbancy at 260 mμ was taken as 20 cm^2/mg. The optical density, taking into account the hyperchromic shift, was converted to nucleotide equivalents assuming a molar extinction coefficient of 10,000. Unless otherwise mentioned DNA concentration will be expressed in moles-nucleotide per milliliter. The DNA preparation used in the experiments described in this report was at a concentration of 2.1 μmoles/ml (3.86 $\times$ 10^5 cpm/μmole). Dilutions for use in the various assays were made in 0.01 M Tris (pH 7.5)–0.02 M NaCl.

Enzyme Assays. The deoxyribonuclease assay measures the conversion of native or denatured [^{3}H]DNA into acid-soluble fragments. The incubation mixture (0.3 ml) contained 36 μmoles of Tris (pH 7.2), 1.2 μmoles of $MgCl_2$, 8.4 mμmoles of [^{3}H]T5 DNA, and various amounts (0.005–0.200 ml) of enzyme solutions. After incubation at 37° for 60 min, 0.2 ml of a solution of DNA (2.5 mg/ml) was added as "carrier," followed by 0.5 ml of cold 3.5% perchloric acid. After 5 min in an ice bath, the mixture was centrifuged for 15 min at 5000 rpm in the Sorvall centrifuge; 0.2 ml was removed and added to 10 ml of Bray's (1960) solution and the radioactivity was counted in a Packard Tri-Carb or Beckman LS-100 scintillation counter. The supernatant obtained from control incubations (enzyme omitted) contained approximately 0.3% of the added radioactivity. A unit of enzyme activity was defined as that amount causing the production of 10^4 cpm (0.026 μmole) of acid-soluble ^{3}H in 60 min. The activity made acid soluble was proportional to enzyme concentration at levels of from 0.01 to 0.11 unit.

Ribonuclease activity was determined in essentially the same manner as DNase activity except that [^{3}H]RNA was used as substrate and EDTA replaced $MgCl_2$. The [^{3}H]RNA, extracted from λ-infected *E. coli*, was supplied by Dr. M. Feiss and was at a concentration of 0.04 μmole of nucleotide/ml (1.4 $\times$ 10^6 cpm/μmole). A unit of activity was defined as that amount causing the production of 0.026 μmole of acid-soluble ^{3}H in 60 min.

Heat-denatured DNA was obtained by heating a DNA solution (less than 100 μg/ml) for 10 min at 100°, followed by immediate cooling in an ice bath.

Protein was determined by the method of Lowry *et al.* (1961). Phosphate was measured by the method of Chen *et al.* (1956) as modified by Ames and Dubin (1960).

Thin-layer chromatography was done on layers of polyethyleneamine cellulose 0.5 mm thick and developed in 0.5 M sodium formate (Randerath and Randerath, 1967).

Purification of Yeast Endonuclease. All steps in the purification, unless otherwise indicated, were carried out at 0–4°.

Growth of Cells and Preparation of Cell-Free Extracts. The cells were grown in 4–6-l. flasks at 30° and harvested by centrifugation at different stages of growth. The yield of enzyme varied depending on the stage of growth and the kind of medium in which the cells were grown. Preliminary trials indicated that yields were higher in SC or MA medium, and routinely strain 9-D1 was grown in MA medium, while C252(2d) was grown in SC medium. Cell-free extracts were prepared by lysing spheroplast preparations or by agitation in a glass bead shaker, as described below. Stationary phase cells resistant to the Glusulase preparation were extracted with the glass bead shaker.

Lysis of Spheroplasts. Spheroplasts of exponentially growing cells were prepared by a slight modification of the procedure of Duell *et al.* (1964). To every 2–4 g of cells (wet weight) a solution containing 10 ml of 1 M sorbitol, 0.1 M citrate–phosphate buffer (pH 5.8), 0.03 M 2-mercaptoethylamine-HCl, 4 $\times$ 10^{-4} M EDTA, and 0.2 ml of Glusulase was added; after thorough mixing the suspension was incubated at 37° for 30–120 min. After spheroplast formation was judged maximal, the suspension was centrifuged for 5 min at 1000 rpm. The supernatant was poured off, and the pellet was washed once with 10 ml of 1 M sorbitol. The spheroplast pellet was lysed by addition of 10 ml of 0.01 M Tris (pH 7.4)–0.002 M phenylmethylsulfonyl fluoride with thorough mixing. Phenylmethylsulfonyl fluoride was added routinely as a protease inhibitor (Schulze and Colowick, 1969). Sodium deoxycholate and Brij-58 were added to a final concentration of 0.5% and the mixture was kept in an ice bath for another 20 min. Neither phenylmethylsulfonyl fluoride, sodium deoxycholate, or Brij-58 reduced the activity of the yeast endonuclease and all appeared to increase the recovery.

Extraction with a Glass Bead Shaker. Extracts were made with the B. Braun MSK mechanical cell homogenizer equipped with a liquid CO_2 tank to maintain low temperatures during disruption. Ten grams (wet weight) was suspended in 10 ml of 0.01 M Tris (pH 7.4)–0.002 M phenylmethylsulfonyl fluoride in the 40-ml glass flask provided with the homogenizer. Approximately one to two volumes of 0.45–0.50-mm diameter glass beads were added. CO_2 was allowed to circulate around the flask for 30 sec. The MSK (set at 4000 rpm) was turned on and off alternatively for 15 sec until the total agitation time was approximately 1–15 min. This normally resulted in 90–99% breakage of cells. After agitation the beads were allowed to settle, and the broken cell suspension was poured off. The beads were washed twice in 10 ml of the same buffer, and sodium deoxycholate and Brij-58 added to a concentration of 0.5%.

The spheroplast or glass bead extract was centrifuged for 10 min at 5000 rpm. The supernatant was further centrifuged for 20 min at 10,000 rpm. Ribosomes were sedimented by centrifugation for 1 hr at 60,000 rpm in the 65 rotor of the Beckman L2-65 ultracentrifuge. The ribosomal pellet was washed by suspending in the same buffer and resedi-

[1] TM buffer = 0.01 M Tris–0.01 M Mg^{2+} (pH 7.4).

TABLE I: Purification of Yeast Deoxyribonuclease (Endonuclease A).

	Protein (mg/ml)	Sp Act. (Units/ mg of Protein)	Purificn	Total Act.	Yield (%)
Crude extract	12.1	0.10	1	108	100
$(NH_4)_2SO_4$	9.25	0.20	2	18.7	17
CM-cellulose	0.18	31.3	313	56.3	52

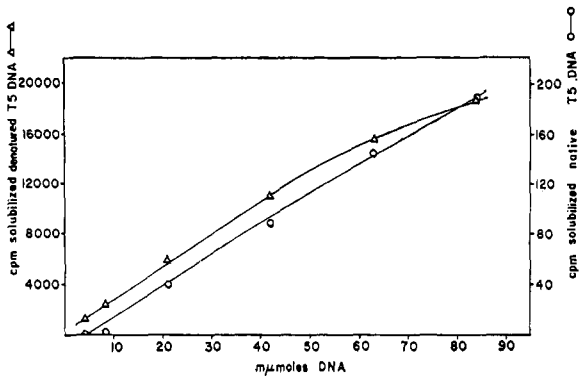


FIGURE 1: Rate of attack of yeast deoxyribonuclease on native and heat-denatured DNA. The incubation mixture (0.3 ml) contained 36 μmoles of Tris buffer (pH 7.2), 1.2 μmoles of $MgCl_2$, 0.112 unit of CM-cellulose fraction, and varying amounts of substrate. Incubation was for 60 min at 37°. Acid-soluble radioactivity was determined as described in Methods.

menting as before, and the ribosomal washings were combined (crude extract).

Ammonium Sulfate Fractionation. Fractionation with ammonium sulfate gave somewhat variable results, but normally 80–90% of the recoverable activity occurred between 0.2 and 0.5 saturation and the solution was allowed to stand for 25 min after the ammonium sulfate had dissolved. The suspension was centrifuged for 20 min at 10,000 rpm. The precipitate was discarded and ammonium sulfate was added to the supernatant to half-saturation. After centrifugation, the resulting precipitate was dissolved in one-fifth volume (crude extract) in 0.01 M sodium maleate buffer (pH 5.8) and dialyzed overnight against 100 volumes of the same maleate buffer.

CM-cellulose Chromatography. A column (16 × 2 cm) was prepared and equilibrated with 0.01 M sodium maleate (pH 5.8). The dialyzed ammonium sulfate fraction was applied to the column, and the absorbent was washed with 10 ml of maleate buffer. A linear gradient of elution was applied with 0 and 0.3 M NaCl (pH 5.8), as limiting concentrations. The total volume of the gradient was 220 ml; the flow rate was 35 ml/hr and 4-ml fractions were collected. The peak of activity appeared approximately at 0.1 M NaCl. The peak fractions were pooled and dialyzed against 0.01 M maleate buffer (pH 5.8). Enzyme at this level of purification was used in all the experiments to be described.

Results

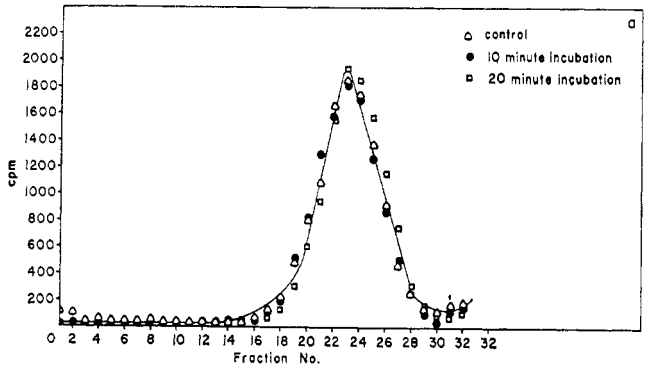
The results of a purification run for endonuclease A are shown in Table I. The ammonium sulfate step was in general the most variable step in the procedure, and the purification achieved in this step varied from 0.5- to 3-fold. As shown in Table I the recovery in the CM-cellulose fraction was greater than in the ammonium sulfate fraction. This suggests that enzymatic activity in the ammonium sulfate fraction was being masked, perhaps by inhibitors, and that these are removed by the CM-cellulose. Although the ammonium sulfate step did not result in a significant purification, it did increase the overall yield of enzyme and decreased the amount of RNase contamination in the CM-cellulose fraction.

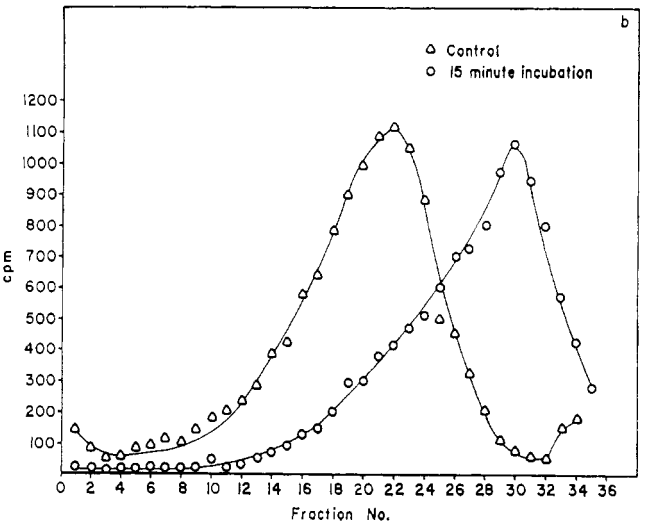
Studies on Enzymatic Mechanism. HYDROLYSIS OF NATIVE *vs.* DENATURED DNA. Yeast endonuclease A hydrolyzes heat-denatured DNA at about 100 times the rate observed with intact, native DNA (Figure 1). The residual activity on double-stranded DNA may represent the effects of a contaminating enzyme, since preliminary work also indicates the presence of another enzyme in yeast extracts with a preference for double-stranded DNA.

Sucrose gradient analyses of native DNA treated with the enzyme indicated furthermore that yeast endonuclease A does not make single-stranded scissions in double-stranded DNA. Native [^{3}H]T5 DNA was incubated for 10 and 20 min with an amount of enzyme which, when incubated with heat-denatured DNA, rendered 4 and 7%, respectively, of the denatured DNA in the assay volume acid soluble. The DNA was then heat denatured and centrifuged on neutral sucrose gradients (Studier, 1965). The results (Figure 2a) show no reduction in the average single-stranded molecular weight of the enzyme-treated double-stranded DNA as compared with an untreated control. In contrast, when hear-denatured DNA was used as substrate instead, a readily detected drop in molecular weight was seen (Figure 2b). In comparison to the small amount of DNA made acid soluble, such a large drop in molecular weight can only be accounted for by endonucleolytic cleavage.

The hydrolysis of single-stranded DNA was also monitored by the endonuclease assay of Geiduschek and Daniels (1965). Their assay is based on the property of nitrocellulose membrane filters to retain only relatively large polynucleotide chains of denatured DNA, and should therefore serve as a fairly sensitive assay of endonuclease activity. Hence, as shown in Figure 2c, with 0.056 unit of CM-cellulose fraction 19% of the DNA was made acid soluble in 15 min while there was a corresponding loss of 45% of the counts bound to the filter. When the amount of enzyme is increased to 0.56 unit such that 45% of the DNA counts are made acid soluble, 95% of the radioactivity is not retained by the filter. These results suggest that the enzyme does not act by cleaving short fragments from the ends of DNA chains but rather makes (probably random) nicks throughout the molecule.

The substrate specificity of endonuclease A was checked by the spheroplast infectivity assay of Guthrie and Sinsheimer (1963). The DNA (2 μg) from phage φX174 (circular, single stranded) and φX174 RF (circular, double stranded)





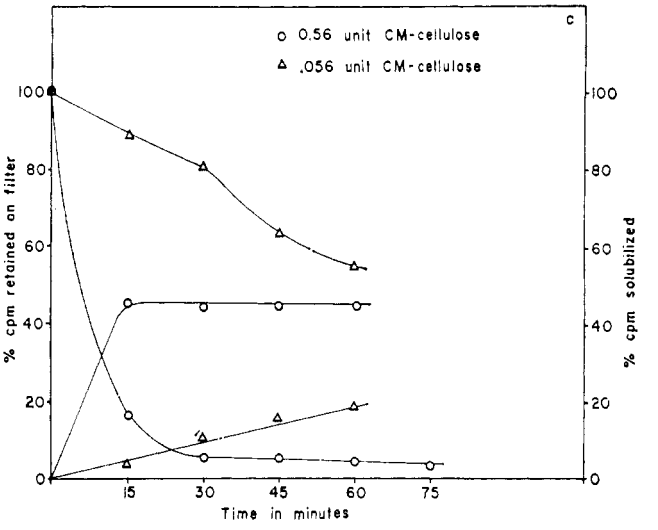


FIGURE 2: DNA analtyical studies. (a) Sucrose gradient analysis of double-stranded DNA treated with yeast deoxyribonuclease. The incubation mixture (0.1 ml) contained 12 μmoles of Tris buffer (pH 7.2), 0.4 μmole of $MgCl_2$, 16.8 mμmoles of [^{3}H]T5 double-stranded DNA, and 0.028 unit of CM-cellulose fraction. After 10 and 20 min at 37°, the mixture was heated 10 min at 100°, then cooled rapidly in an ice bath. The mixture was then layered on a 5-ml 5–20% sucrose gradient containing 0.1 M Tris (pH 7.9)–0.01 M NaCl–0.001 M EDTA. Centrifugation was at 32,000 rpm for 5 hr in the Spinco SW50L rotor. Ten drop fractions were collected on GF/C Whatman glass fiber filters and the radioactivity was counted on a Beckman LS-100 scintillation counter. (b) Sucrose gradient analysis of single-stranded DNA treated with yeast deoxyribonuclease. The assay mixture was as in part a except that 16.8 mμmoles of heat-denatured [^{3}H]T5 DNA was used as substrate. After 15 min at 37°, 0.01 ml of 0.2 M EDTA was added, and the mixture was heated for 10 min at 60°. Centrifugation was as in part a. (c) Endonuclease assay of Geiduschek and Daniels. Assay mixture (0.3 ml) contained 36 μmoles of Tris (pH 7.2), 1.2 μmoles of $MgCl_2$, 8.4 mμmoles of heat-denatured [^{3}H]T5 DNA, and either 0.056 or 0.56 unit of CM-cellulose fraction. At the times indicated the reaction was stopped by the addition of 10–15 ml of 0.5 M KCl–0.01 M Tris–0.01 M EDTA (pH 7.5). The solution was filtered through nitrocellulose membranes (Schleicher & Schuell type B-6) with approximately 50 ml of the KCl–Tris–EDTA buffer. The filters were dried and counted. Duplicate incubations were run to determine acid-soluble fragments.

were incubated in a standard assay solution with 0.028 unit of CM-cellulose fraction for 0 and 40 min at 37°. The reaction mixture was diluted 1:1000 into 0.05 M Tris (pH 8.2)–0.001 M EDTA, and the intactness of the DNA was measured by infectivity to spheroplasts.[2] A single break will make the DNA noninfective. If the activity of the enzyme is measured by the ratio of plaque-forming units at 40 min to those at 0 min $(S_{40})/(S_0)$, then the following is obtained: (a) for φX174 single-stranded form $(S_{40})/(S_0) = 0.0008$, and (b) for the φX174 RF form $(S_{40})/(S_0) = 0.6$. Hence, this assay indicates that endonuclease A has approximately a 1000-fold preference for single-stranded DNA over double-stranded DNA.

LIMITED HYDROLYSIS. In Figure 3a is shown the rate of hydrolysis of heat-denatured DNA. The amount of radioactivity made acid soluble was never 100% of that added to the assay mixture. Although the amount varied with the preparation and the substrate, the values so far determined have not exceeded 60%. As shown in Figure 3b, the addition of 0.28 unit more enzyme after the acid-soluble limit is reached does not result in further degradation. On the other hand, as shown in Figure 3c, the addition of more substrate does lead to more acid-soluble counts. To test whether the products of hydrolysis acted as inhibitors of enzyme action, portions of various limit digests were added to normal assay mixtures. In all cases tested the digest products had no inhibitory effect on the enzyme. These results are consistent with the conclusion that the action of the enzyme is endonucleolytic and show that the acid-soluble limit is reached not because of enzyme inactivation or inhibition, but that the oligonucleotides formed are probably resistant to the action of this enzyme. The average length of these fragments has been estimated by determining the fraction of the total phosphate in an assay mixture which is sensitive to the action of *E. coli* alkaline phosphatase. The results are shown in Figure 4, where both the phosphatase-sensitive phosphate and the average chain length (in nucleotides) are plotted as a function of time. At the end of the incubation period a maximum of 58% of the DNA in the assay mixture had been rendered acid soluble. It should be remarked that the average chain length after extensive hydrolysis was found to be somewhat variable and depended on the substrate

[2] The help of Dr. E. T. Young in whose laboratory the spheroplast assays were done is greatefully acknowledged.

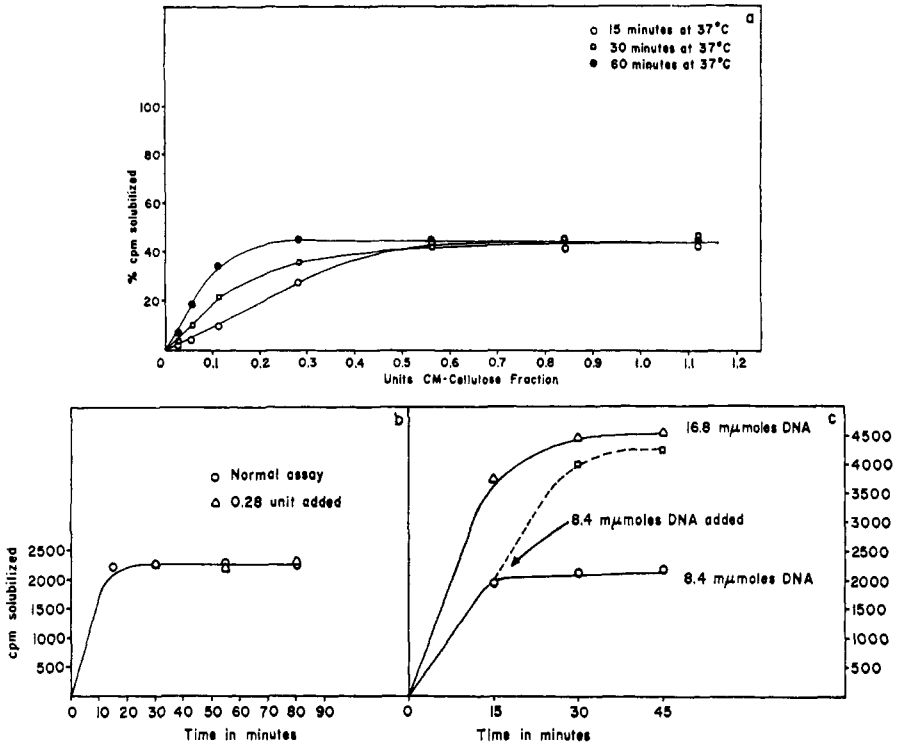


FIGURE 3. (a) Rate of hydrolysis of heat-denatured DNA. The incubation mixture (0.3 ml) contained 36 μmoles of Tris buffer (pH 7.2), 1.2 μmoles of $MgCl_2$, 8.4 mμmoles of denatured [^{3}H]T5 DNA, and varying amounts of CM-cellulose fraction. Incubation was at 37° for the times indicated. (b) Inability of added enzyme to increase amount of acid-soluble products. The incubation mixture (2.1 ml) contained 252 μmoles of Tris buffer (pH 7.2), 8.2 μmoles of $MgCl_2$, and 58.8 mμmoles of denatured [^{3}H]T5 DNA. Incubation was at 37°. At 15 and 30 min 0.3 ml was removed and acid-soluble products were measured as described in Methods. In addition, at 30 min the incubation mixture was divided into two portions: to one an additional 0.280 unit of CM-cellulose fraction was added and incubation was continued at 37°. At the indicated times 0.3 ml was removed from both portions and acid-soluble radioactivity determined. (c) Increase in acid-soluble radioactivity with added substrate. The incubation mixture (0.3 ml) contained 36 μmoles of Tris buffer (pH 7.2), 1.2 μmoles of $MgCl_2$, 0.56 unit of CM-cellulose, and 8.4 mμmoles of denatured [^{3}H]T5 DNA. Two identical incubation mixtures were run simultaneously. After 15 min at 37° an additional 8.4 mμmoles of DNA was added to one assay mixture. Acid-soluble radioactivity was then determined at 30 min. A control run with 16.8 mμmoles of DNA was also run simultaneously.

and enzyme preparation. However, all values obtained to date have in the range of four to eight nucleotides.

To check for the possibility that the enzyme might also exhibit exonucleotic activity, incubation mixtures were subjected to thin-layer chromatography to test for the presence of mononucleotides. In an acid-soluble limit digest 45% of the radioactivity was rendered acid soluble; but as indicated in Table II a maximum of 1.5% could be found as TMP and dCMP, suggesting that yeast endonuclease A has no significant exonucleolytic activity.

Properties of the Purified Enzyme. CATION REQUIREMENTS. Inhibition and activation of enzymic activity was measured by adding various agents as reported in Table III. It can be seen that the enzyme requires Mg^{2+} or Mn^{2+}, and that Ca^{2+} will not replace either cation. Activity was also completely inhibited by extremely low levels of EDTA suggesting that the residual activity without Mg^{2+} is the result of traces of the metal ion present in the incubation mixture.

SPECIFICITY. Incubation of digests of yeast endonuclease A containing 2.1 and 19.1% acid-soluble ^{3}H with excessive amounts of snake venom phosphodiesterase results in a complete conversion of radioactivity into acid-soluble form. In contrast, incubation of similar digests with excessive amounts of bovine spleen phosphodiesterase results in only a 10% increase in acid-soluble counts. Since snake venom

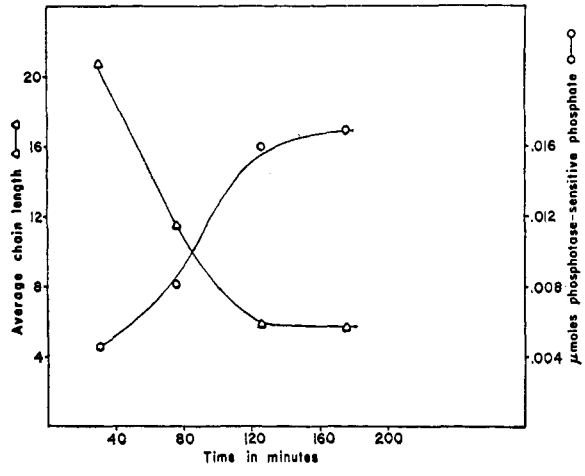


FIGURE 4: Phosphatase-sensitive phosphate and average chain length. To an assay volume (1 ml) containing 100 μmole of Tris buffer (pH 7.2), 5 μmoles of $MgCl_2$, 0.634 μmoles of denatured calf thymus DNA, 0.56 unit of CM-cellulose fraction was added, and the mixture was incubated at 37°. At the times indicated 0.12-ml aliquots were removed, heated 10 min at 60° to inactivate the deoxyribonuclease, and added to the alkaline phosphatase assay mixture (0.3 ml) containing 32 μmoles of Tris (pH 7.9), 1.8 μmoles of $MgCl_2$, and 3.3 units of *E. coli* alkaline phosphatase. After 60 min at 37°, P_i was determined.

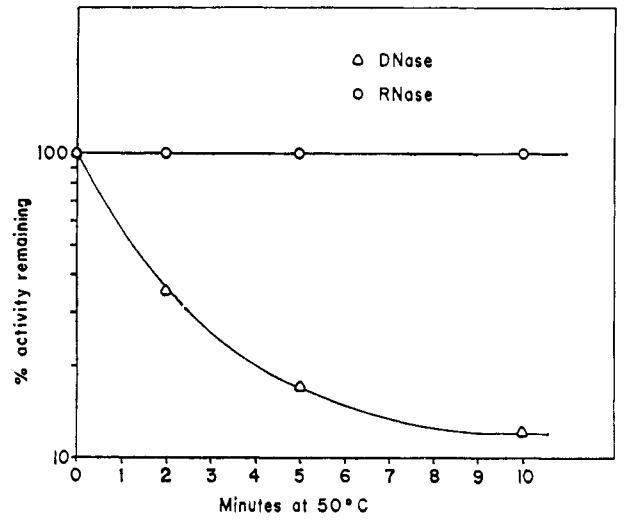


FIGURE 5: Relative heat inactivation of DNase and RNase activities of CM-cellulose fraction. A portion of CM-cellulose fractions was heated at 50°. At the times indicate aliquots (0.28 unit) were removed and assayed for DNase–RNase activity.

phosphodiesterase requires free 3′-hydroxyl end groups, this indicates that the reaction products of yeast endonuclease A are 5′-phosphate-terminated oligonucleotides.

RIBONUCLEASE ACTIVITY. The CM-cellulose fraction contains an amount of ribonuclease activity equal to approximately 20% of its deoxyribonuclease activity. The amount of ribonuclease activity varies according to the preparation but there are two lines of evidence to support the view that the two activities are due to two physically different proteins: (a) on heating at 50°, the DNase activities show different rates of inactivation (Figure 5); (b) the deoxyribonuclease activity shows a requirement for either Mg^{2+} or Mn^{2+} and is completely inhibited by low levels of EDTA, whereas the RNase activity does not require added cations, and is, in fact, slightly activated by 0.0033 M EDTA, and inhibited by 0.0033 Mg^{2+}.

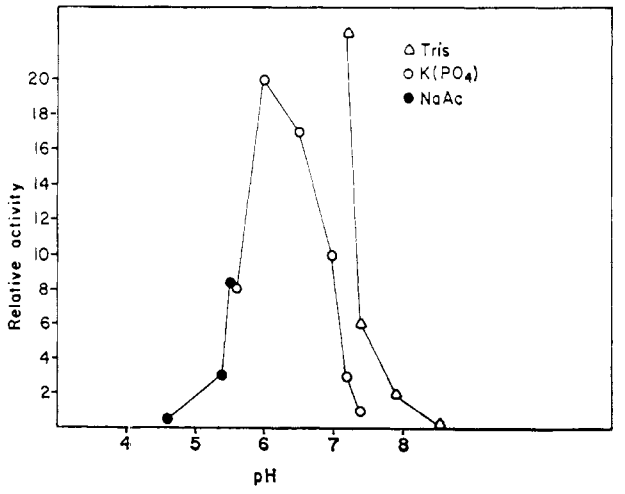


FIGURE 6: pH profile of yeast deoxyribonuclease activity. All incubation mixtures contained 36 μmoles of appropriate buffer and 0.28 unit of CM-cellulose fraction. Incubation was at 37° for 60 min.

TABLE II: Thin-Layer Chromatography of DNase Incubation Mixtures.[a]

	Radioactivity (%)
dCMP	0.8
TMP	0.7
Total	1.5
Acid-soluble products	50

[a] Incubation mixtures (0.3 ml) contained 36 μmoles of Tris (pH 7.2), 1.2 μmoles of $MgCl_2$, 0.56 unit of CM-cellulose fraction, and 25.2 mμmoles of [^{3}H]T5 heat-denatured DNA. After 2 hr at 37° 50 μl of the incubation mixture was spotted on the thin-layer plate. Molar equivalents of TMP and dCMP were spotted to run as controls, and the plate was developed for 4 hr in 0.5 M sodium formate. The radioactivity recovered from positions corresponding to TMP and dCMP is expressed in per cent of the activity spotted on the plate.

pH OPTIMUM. A pH profile is shown in Figure 6. The existence of two pH optima could suggest the presence to two enzymatic activities with very similar substrate requirements. However, the activities speaking at pH 6.0 and 7.2 cochromatograph on CM-cellulose. Attempts to resolve the two activities on the basis of cation or substrate requirements have not been successful. At both pH's Ca^{2+} will not replace Mn^{2+} or Mg^{2+}. Furthermore, attempts to resolve the two activities on DEAE-cellulose, hydroxylapatite, or on sucrose gradients have also not been successful. These

TABLE III: Inhibitors and Activators of Yeast Endonuclease A.

Addition	Concn (M)	% Act.[a]
Mg^{2+}	0.003	100
	0.0003	67
	0.001	97
	0.01	62
	0	2.3
Mn^{2+} ($-Mg^{2+}$)	0.0033	98
Ca^{2+} ($-Mg^{2+}$)	0.0033	2.3
EDTA ($-Mg^{2+}$)	0.0033	0
	0.00033	0
	0.000033	0
NaH_2PO_4 (+0.004 M Mg^{2+})	0.0033	100
	0.033	100
KH_2PO_4 (+0.004 M Mg^{2+})	0.033	100
	0.0033	100

[a] Incubation mixtures (0.3 ml) contained 36 μmoles of Tris (pH 7.2), 8.4 mμmoles of [^{3}H]T5 DNA, 0.28 unit of CM-cellulose fraction, and the indicated amounts of the cations shown. The values shown are expressed as per cent of the activity observed with 0.004 M Mg^{2+}, *i.e.*, 1.2 μmoles of $MgCl_2$ in the assay mixture.

results suggest strongly that the two activities represent the same enzyme.

STABILITY. The CM-cellulose fraction has shown no significant loss of activity after storage at −20° for 6 months.

Discussion

Yeast endonuclease A purified approximately 300-fold appears to represent a distinct enzymatic activity. It has a requirement for Mg^{2+} and Mn^{2+}, which cannot be replaced by Ca^{2+}, and has a decided preference for single-stranded DNA.

Yeast endonuclease A appears to be similar in its properties to *E. coli* endonuclease IV, which has been purified from T4-infected *E. coli* (Sadowski *et al.*, 1968). A *Neurospora* endonuclease (Linn and Lehman, 1965) also has a similar substrate specificity, but in contrast to yeast endonuclease A, the same protein possesses both DNase and RNase activities. In mammalian tissue none of the known DNases (Lindahl *et al.*, 1969) are similar in substrate requirements to yeast endonuclease A.

Questions regarding the function or functions of yeast endonuclease A are impossible to answer at this time. Nevertheless, a few possibilities suggest themselves. Current models of recombination (Holliday, 1964; Whitehouse and Hastings, 1955) share two features: (1) breakage and reunion of single strands of DNA, and (2) repair of mismatched base pairs within a heteroduplex region of DNA. Yeast endonuclease A could conceivably function in either or both of these capacities. These tentative suggestions indicate that studies which attempt to trace variations in enzyme activity during meiosis or under conditions in which repair processes are known to be taking place may be helpful in elucidating the function of this and other DNA-degrading enzymes. Alternatively, the isolation of DNase-deficient mutants and biochemical characterization of mutants with altered repair or recombinational properties should establish much more conclusively the function of such enzymes. Studies of both kinds are in progress.

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