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# THE NUCLEASES OF YEAST

## I. PROPERTIES AND VARIABILITY OF RIBONUCLEASES

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SUMMARY

1. Nucleases are located in both ribosomes and supernatant of the yeast, *Saccharomyces fragilis* × *Saccharomyces dobzhanskii*. Ribosomes prepared from either exponentially growing cells or stationary cells exhibit nuclease activity with a pH optimum of around 7 which is not inhibited by 0.02 M EDTA. Polyvinylsulfate inhibits only at high concentrations. Nuclease activity in the 105 000 × *g* supernatant from exponentially growing cells has a pH optimum of around 8 and is severely inhibited by EDTA and polyvinylsulfate. In contrast, supernatant nuclease from stationary cells has a pH optimum of around 6 and is only partially inhibited by EDTA.

2. Supernatant nuclease I and II from logarithmic cells have been partially purified. Their pH optimums are 7.4 and 7.6, respectively. Both nucleases are inhibited by EDTA, $Zn^{2+}$, polyvinylsulfate and phosphate. The inhibition of EDTA is reversed by the addition of $Mg^{2+}$. Polyribonucleotides are hydrolysed to di- and trioligonucleotides with 5'-phosphomonoester end groups by both nucleases.

## INTRODUCTION

There is evidence that several distinguishable ribonucleic acid degrading mechanisms exist in a single cell. There has been extensive speculation that these enzymes may be involved in destruction of messenger RNA (mRNA)[1], removal of ribosomes during differentiation[2], and modulation of protein synthesis by destruction of specific transfer RNA (tRNA) species[3,4]. The research published to date has not proven any specific nuclease to have one of these roles. However, continued research on identification of the number, kind, localization, and specificity of RNA degrading enzymes may provide a basis for understanding these processes. There have

Abbreviations: tRNA, transfer RNA; mRNA, messenger RNA; poly(A), polyadenylic acid; poly(U), polyuridilic acid; TMK buffer, 0.05 M Tris buffer (pH 7.6), 0.005 M magnesium acetate and 0.01 M KCl.

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been extensive descriptions of the purified enzymes from *Escherichia coli*. In this paper, we describe the location of nucleases in yeast, changes in the activity of these enzymes during different stages of growth, and sufficient purification or separation that the distinguishing specificity differences can be demonstrated. One of these nucleases is described in detail and methods for its purification on preparative scale are presented.

A ribonuclease which is bound to ribosomes in yeast cell extracts has been purified and its specificity described[5]. DANNER AND MORGAN[6] found that the nucleases free in the cytoplasm did not increase during the period in which ribosomal bound nuclease increased. Our work demonstrates that division of ribonucleases into these two classes is inadequate to describe the variety of distinguishable specificities and separable activities that exist in a yeast cell. These researches are not yet capable of defining whether the enzymes described account for all of the ribonucleic acid degrading mechanisms in yeast. Specificity of one of the supernatant nucleases is described in detail in the accompanying paper.

## MATERIALS AND METHODS

Yeast RNA was obtained from Pabst Laboratories. The yeast RNA was purified further by phenol, then methoxyethanol extraction, alcohol precipitation, dialysis against 0.1 M $NH_4HCO_3$ and lyophilization. tRNA was purchased from General Biochemicals. Polyadenylic acid (poly (A)) and polyuridylic acid (poly (U)) were obtained from Miles Chemical Cy. Crystalline bovine plasma albumin, highly polymerized DNA and DEAE-cellulose were obtained from California Corporation for Biochemical Research. Partially degraded DNA was obtained from Nutritional Biochemical Corp. Calcium bis (*p*-nitrophenyl) phosphate and *p*-nitrophenyl phosphate products from Sigma Cy. Sephadex G-200 was obtained from Pharmacia, Uppsala. were Superbrite glass beads were purchased from Minnesota Mining and Manufacturing Company.

### *Growth of cells and preparation of cell-free extracts*

A hybrid yeast *(Saccharomyces fragilis* × *Saccharomyces dobzhanskii)* was used in these experiments. The cells were grown essentially as described by BRETTHAUER *et al.*[7] and harvested by centrifugation at different stages of growth. The cells were washed twice with cold TMK buffer (0.05 M Tris buffer (pH 7.6), 0.005 M magnesium acetate and 0.01 M KCl) and then disrupted by grinding with twice their weight of acid-washed sand for 5 min in a mortar placed in a salt–ice bath at −5°. The mixture was extracted with 2–3 vol. of buffer for 1–2 min and then centrifuged at 10 000 × *g* for 10 min to remove the sand and large debris. The supernatant fluid was further centrifuged twice at 20 000 × *g* for 20 and 30 min respectively. Ribosomes were then sedimented at 105 000 × *g* for 2 h in a Spinco Model-L centrifuge. The ribosomal pellet was suspended in the TMK buffer and washed by resedimenting at 105 000 × *g* for 2 h. The supernatant solution and the ribosomes could be stored at −20° for several months with no loss in nuclease activity.

### *Release of nuclease from ribosomes*

Ribosomal nuclease was released by the method of CURRY AND HERSH[8].

Ribosomes were suspended in the TMK buffer and an equal volume of 4 M LiCl solution was added. After standing at 4° for 16 h, the precipitate was removed by centrifugation at 20 000 × *g* for 15 min. The supernatant solution was dialyzed overnight in the cold against three changes of the TMK buffer. The precipitate which appeared was removed by centrifugation and the supernatant was used as a ribosomal nuclease unless otherwise mentioned.

*Assay of enzymes*

The activity of nuclease was determined by a slight modification of the procedure of ANFINSEN *et al.*[9]. The reaction mixture, unless otherwise stated, contained: 0.1 ml of 1% solution of purified yeast RNA; 0.2 ml of 0.5 M Tris buffer (pH 8.0); and 0.1 ml of enzyme in dilute TMK buffer except when the experiment was a test of the effect of magnesium.

After incubation at 37° for 60 min, 0.4 ml of 0.25% uranyl acetate in 2.5% perchloric acid was added and the mixture was kept at 0° for 15 min. The precipitate was centrifuged in the cold; 0.2 ml of the clear supernatant was diluted to 4.0 ml with distilled water, and the absorbance of this solution was read at 260 mμ against a zero-time blank. A unit of nuclease was defined as the amount of enzyme which, under the above conditions, gave an absorbance change of 0.01. The assay was linear up to 0.6 absorbance. The specific activity is expressed in units/mg of protein.

Alkaline phosphatase was assayed by a modification of the procedure of KOERNER AND SINSHEIMER[10]. The reaction mixture contained: 0.2 ml of 2 mM *p*-nitrophenyl phosphate; 0.7 ml of 0.25 M Tris buffer (pH 8.0); and 0.1 ml of enzyme solution. After incubation at 37° for 30 min, 1.0 ml of 0.5 M NaOH was added and the absorbance was measured at 400 mμ. A unit of phosphatase is defined as the amount of enzyme which gives an absorbance of 0.1. Alkaline phosphatase was also assayed by the following method. 0.1 ml of 10 mM 5'-AMP or 3'-AMP and 0.2 ml of 0.5 M Tris buffer (pH 8.0) were incubated with 0.1 ml of the enzyme solution at 37° for 30 min and the orthophosphate liberated was determined by the method of GOMORI[11].

Phosphodiesterase activity was assayed using calcium bis(*p*-nitrophenyl) phosphate as substrate. 1 ml of 1 mM bis(*p*-nitrophenyl) phosphate, 0.5 ml of 0.5 M Tris buffer (pH 8.0) containing 0.01 M $MgSO_4 \cdot 7\ H_2O$ and 0.5 ml of the enzyme solution were mixed. After incubation at 37° for 5 h, the reaction was stopped by addition of 2.0 ml of 0.5 M NaOH and the precipitate formed was removed by centrifugation. The absorbance of the supernatant was measured at 400 mμ.

Deoxyribonuclease activity was determined in essentially the same manner as nuclease activity except that 1% DNA solution was used as the substrate.

Protein content was estimated by the phenol procedure of LOWRY *et al.*[12].

## RESULTS

*Changes in nucleases at different stages of growth*

The properties of nucleases were investigated as a function of the growth phase. The extracts from logarithmically growing cells and stationary cells were fractionated by centrifugation into the ribosomal and soluble components as described in MATERIALS AND METHODS. The ribosomes and the supernatant thus obtained were used as

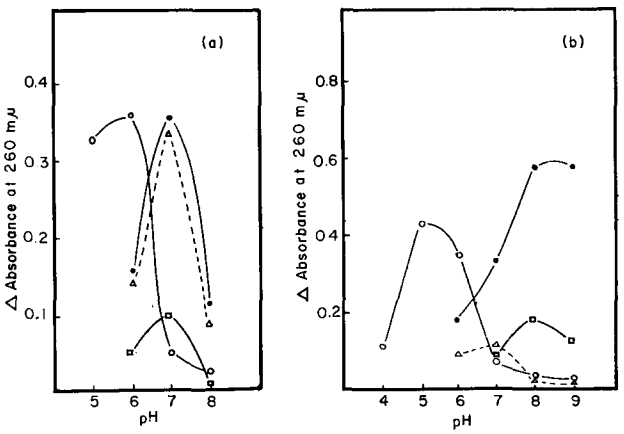


Fig. 1. Effects of pH, EDTA and urea on the hydrolysis of RNA by the ribosomal (a) and supernatant (b) nucleases obtained from logarithmic cells. RNA was hydrolyzed for 3 h at 37° and the acid-soluble products were measured as in the standard assay. The ribosome suspension and the supernatant contained 3 mg/ml and 8 mg/ml protein, respectively. ●—●, yeast RNA hydrolysis in the absence of EDTA and urea; △- - - -△, yeast RNA hydrolysis in the presence of 0.02 M EDTA; ○—○, yeast RNA hydrolysis in the presence of 4 M urea; □—□, tRNA hydrolysis in the absence of EDTA and urea.

the enzyme solutions and nuclease activity was measured as a function of pH in the presence or absence of EDTA and urea.

The pH optimum and inhibition by EDTA of the supernatant nucleases from logarithmic cells differ from stationary cells, but the ribosomal nucleases from logarithmic and stationary cells showed no such differences (see Figs. 1 and 2). Supernatant nucleases from logarithmic and stationary cells also differ in their activity on tRNA. This may reflect a difference in specificity of the two enzymes.

TABLE I

RATE OF DEGRADATION OF SYNTHETIC POLYNUCLEOTIDES AND YEAST RNA

Each substrate was degraded by supernatant nuclease for 20 min at 37° in Tris buffer, pH 7.6, containing 0.005 M $Mg^{2+}$ and degraded by ribosomal nuclease for 60 min at 37° in Tris buffer, pH 7.6. After incubation, the acid-soluble nucleotide formed was determined as described in *Assay of enzymes*. Protein contents of supernatant nuclease and ribosomal nuclease solution were 0.44 mg/ml and 2.5 mg/ml, respectively.

| *Nuclease* | *Substrate* | *% of degradation* | *Relative activity** |
|---|---|---|---|
| Supernatant | poly(A) | 79.0 | 100 |
| Supernatant | poly(U) | 27.8 | 35.1 |
| Supernatant | yeast RNA | 20.0 | 25.3 |
| Ribosomal | poly(A) | 52.0 | 100 |
| Ribosomal | poly(U) | 54.0 | 103 |
| Ribosomal | yeast RNA | 67.0 | 129 |

* Data are expressed in values relative to poly(A) degradation.

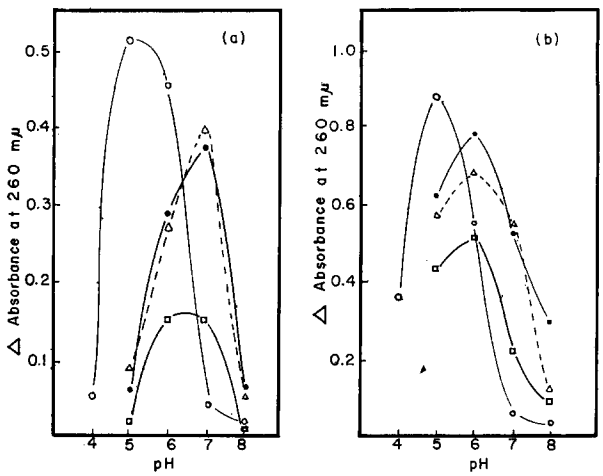


Fig. 2. Effects of pH, EDTA and urea on the hydrolysis of RNA by the ribosomal (a) and supernatant (b) nucleases obtained from stationary cells. RNA was hydrolyzed at each pH for 3 h at 37° and the acid-soluble products were measured as in the standard assay. The ribosome suspension and the supernatant contained 2.2 mg/ml and 8.5 mg/ml protein, respectively. ●—●, yeast RNA hydrolysis in the absence of EDTA and urea; △- - - -△, yeast RNA hydrolysis in the presence of 0.02 M EDTA; ○—○, yeast RNA hydrolysis in the presence of 4 M urea; □—□, tRNA hydrolysis in the absence of EDTA and urea.

*Degradation of polynucleotides and RNA by the supernatant and ribosomal extracts from logarithmic cells*

The relative rates of degradation of poly(A), poly(U), and yeast RNA by the supernatant and ribosomal extracts from logarithmic cells are compared in Table I. The enzyme fractions used here were from $(NH_4)_2SO_4$ precipitation of the supernatant (the preparation obtained from Step 2 in the purification procedure) and a LiCl extract of ribosomes described in MATERIALS AND METHODS. The supernatant preparation was diluted 1:32 with distilled water and 800 μg/ml solutions of poly (A), poly (U), and yeast RNA were used as a substrate instead of the 1% RNA solution in the standard nuclease assay method.

The data in Table I further differentiate ribonuclease activities in supernatant and ribosomal extract from logarithmic cells.

*Inhibition of supernatant and ribosomal nucleases by polyvinylsulfate and spermidine*

Efforts were made to suppress ribonuclease activity in a cell-free protein synthesis system. The same enzyme preparations as those described above were used except that the supernatant solution was diluted 1:2. Figs. 3 and 4 show the inhibition of ribonuclease activity by polyvinylsulfate and spermidine. Supernatant nuclease activity was markedly inhibited by polyvinylsulfate. On the contrary, ribosomal nuclease was not inhibited at low concentrations of polyvinylsulfate but was inhibited 25% at the concentration of 50 μg/ml and 90% at the concentration of 100 μg/ml. The dependence on polyvinylsulfate concentration probably reflects the presence of contaminating proteins because, as will be seen in Table III, inhibition of purified supernatant nuclease occurs at very low levels of polyvinylsulfate.

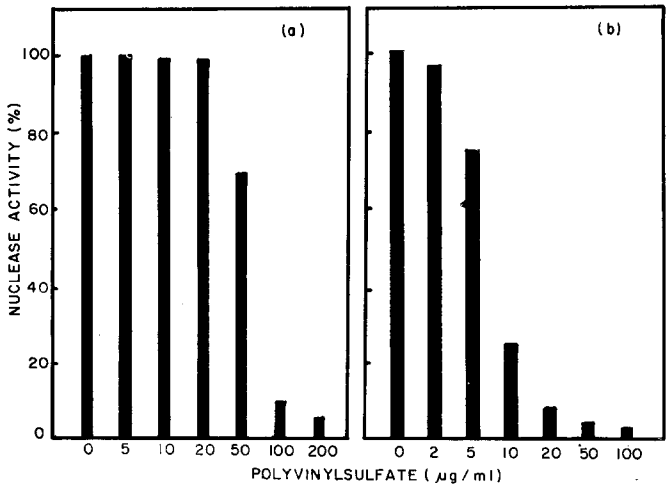


Fig. 3. Effect of polyvinylsulfate on hydrolysis of RNA by both ribosomal (a) and supernatant (b) nucleases. Protein contents of supernatant nuclease and ribosomal nuclease solution were 7.0 mg/ml and 2.5 mg/ml, respectively. Yeast RNA was used as the substrate and degraded by supernatant nuclease for 60 min at 37° by using Tris buffer, (pH 8.0) containing 0.005 M $Mg^{2+}$ and by ribosomal nuclease for 120 min at 37° by using Tris buffer (pH 7.2).

Spermidine inhibits neither nuclease at a low concentration but inhibits slightly at a high concentration.

*Purification of supernatant nucleases from exponentially growing cells*

All steps were carried out at 4°.

*Step 1: Extraction.* Frozen yeast cell paste (weight of wet cell paste was 400 g before frozen), 800 ml of 0.05 M Tris buffer (pH 7.6) containing 0.005 M $Mg^{2+}$ and

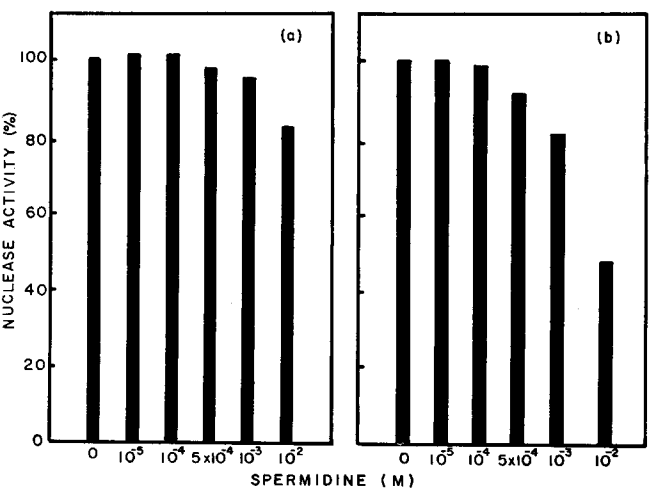


Fig. 4. Effect of spermidine on hydrolysis of RNA by both ribosomal (a) and supernatant (b) nucleases. The conditions of RNA hydrolysis were the same as that described in Fig. 3.

0.01 M KCl, and 500 ml of acid-washed superbrite glass beads (100 $\mu$ in diameter) were added to a colloid mill[13]. The mixture was homogenized for 30 min at 4–7°. After disrupting the cells, the beads were allowed to settle, and the supernatant was decanted. The sediment from the decantation was washed with 200 ml of Tris buffer. The supernatant and the washing were combined and centrifuged at 20 000 × *g* for 30 min in a Servall centrifuge. The supernatant was further centrifuged at 105 000 × *g* for 120 min in a Spinco Model L. The supernatant obtained was used as a crude extract and could be stored at −20° for a month with no loss in activity of the nucleases.

*Step 2: $(NH_4)_2SO_4$ fractionation.* $(NH_4)_2SO_4$, 39.5 g, was slowly added to 210 ml of the extract. The solution was stirred for 180 min, and the small amount of precipitate was removed by 20 min of centrifugation at 25 000 × *g*. Then 39.5 g of $(NH_4)_2SO_4$ was added to the supernatant under conditions similar to the first addition. The precipitate was collected by centrifugation and dissolved in 30 ml of 0.02 M Tris buffer (pH 7.6). The solution obtained was dialyzed overnight in the cold against four changes of 2 l of 0.02 M Tris buffer (pH 7.6).

*Step 3: Gel filtration.* The enzyme solution from Step 2 was chromatographed on a Sephadex G-200 column (19.6 $cm^2$ × 70 cm) which was equilibrated with 0.05 M Tris buffer (pH 7.6). The sample was eluted with the same buffer at a rate of 20 ml/h. 10-ml fractions were collected and protein, nuclease and alkaline phosphatase activity were determined. The elution profile is shown in Fig. 5. The contents of tube 21 through tube 29 were pooled.

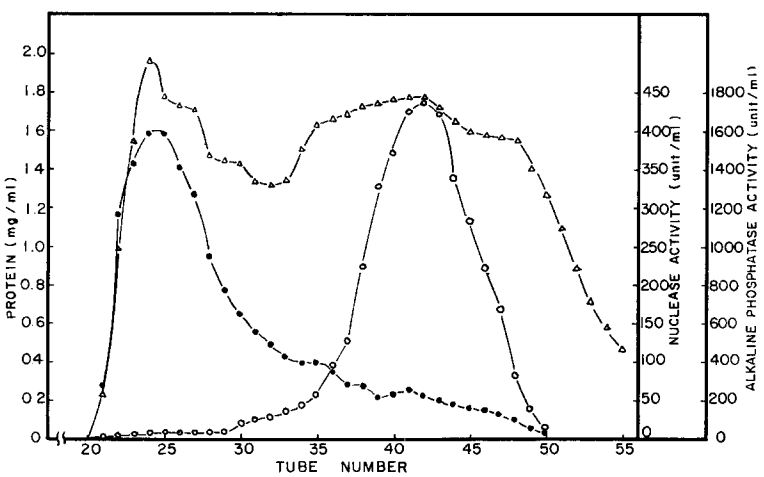


Fig. 5. Distribution of protein, nuclease and alkaline phosphatase activity after gel filtration on Sephadex G-200. The column (70 cm × 5 cm, diameter) was loaded with 36 000 units of nuclease. A 10-ml fraction was collected every 30 min. ●—●, nuclease activity; ○—○, alkaline phosphatase-activity; △—△, protein.

*Step 4: Concentration by $(NH_4)_2SO_4$ precipitation.* The nuclease was precipitated by addition of 54.7 g of $(NH_4)_2SO_4$ to 80 ml of the pooled eluate under conditions similar to those of Step 2. The precipitate was collected by centrifugation for 30 min at 25 000 × *g* and was dissolved in 8 ml of 0.02 M Tris buffer (pH 7.6). The solution was dialyzed overnight against three changes of 2 l of the same buffer.

*Step 5: Chromatography on DEAE-cellulose.* The enzyme solution from Step 4 was loaded on a DEAE-cellulose column (3.1 $cm^2 \times 27$ cm) which was equilibrated with 0.02 M Tris buffer (pH 7.6). A linear gradient was applied with 250 ml of 0.02 M Tris buffer (pH 7.6) in the mixing vessel and 250 ml of 0.4 M KCl in 0.02 M Tris buffer (pH 7.6) in the second container. A flow rate of 16 ml per h was maintained; 4-ml fractions were collected. The results are shown in Fig. 6. Fraction A (tube 55 through tube 75 )and B (tube 107 through tube 120) were pooled and nucleases in these fractions were designated as nuclease I and II, respectively.

*Step 6: Concentration by $(NH_4)_2SO_4$ precipitation.* In this preparation, since protein concentration was low, 0.7 mg bovine plasma albumin was added per ml as a carier. Addition of 41.0 g $(NH_4)_2SO_4$ to 65 ml of Fraction A precipitated nuclease I; 31.5 g $(NH_4)_2SO_4$ to 48 ml of Fraction B precipitated nuclease II. The precipitates were collected by centrifugation for 30 min at 25 000 $\times$ *g*, dissolved in 0.02 M Tris buffer (2.7 ml and 4.5 ml for I and II, respectively), and dialyzed for 16 h against 4 changes of 1 l of the same buffer. Table II gives a summary of the purification in a representative experiment. Nuclease I and II have been purified 15.0-fold with 5.0% yield and 29.5-fold with 7.8% yield, respectively. Unless otherwise mentioned, all experiments described below have been carried out with the same enzyme solutions.

TABLE II

PURIFICATION OF SUPERNATANT NUCLEASES FROM EXPONENTIALLY GROWING CELLS

| *Purification step* | *Volume (ml)* | *Units/ml* | *Protein (mg/ml)* | *Specific activity (units/mg protein)* | *Purification (-fold)* | *Yield (%)* |
|---|---|---|---|---|---|---|
| 1. Extract | 210 | 250 | 7.5 | 33.5 | 1.00 | 100 |
| 2. $(NH_4)_2SO_4$ fraction | 30 | 1200 | 22.0 | 55.0 | 1.64 | 69.0 |
| 3. Gel filtration | 80 | 342 | 1.35 | 255.0 | 7.6 | 52.5 |
| 4. Concentration by $(NH_4)_2SO_4$ | 8 | 2770 | 10.5 | 264.0 | 7.85 | 42.3 |
| 5. DEAE-cellulose chromatography: Nuclease I | 65 | 40.0 | 0.08 | 500 | 15.0 | 4.95 |
| Nuclease II | 48 | 84.5 | 0.085 | 990 | 29.5 | 7.75 |
| 6. Concentration by $(NH_4)_2SO_4$ Nuclease I | 2.7 | 615 | —* | —* | —* | 3.15 |
| Nuclease II | 4.5 | 535 | — | — | — | 4.65 |

* Protein concentration and purification are not meaningful because bovine serum albumin was added to aid in precipitation of dilute solutions.

*Properties of the nuclease I and II*

Several properties of the nucleases were investigated.

*pH optimum:* The activities of nuclease I and II at different pH values were measured in Tris buffers ranging from pH 7.2 to 8.8 and in acetate buffers ranging from pH 5.0 to 5.6. The pH of optimal activity of nuclease I and II were found to be 7.4 and 7.6, respectively.

*Stability:* The purified enzyme preparations have been stored frozen for over 2 months without detectable loss of activity. The enzyme was stable to storage at

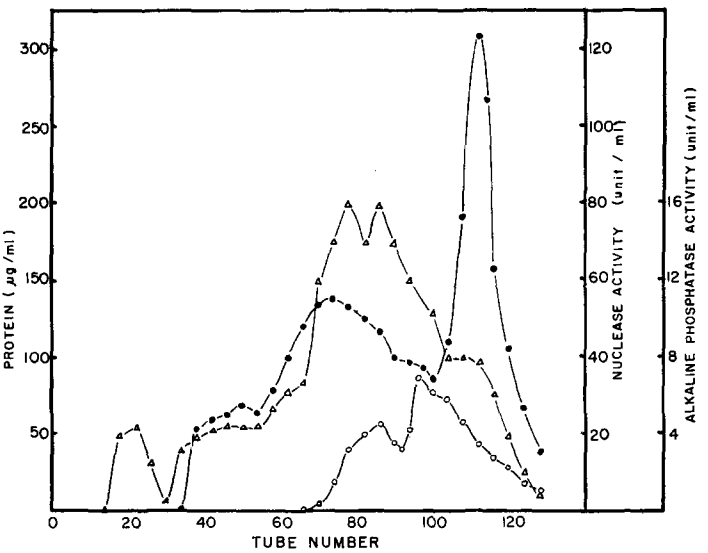


Fig. 6. Chromatography on DEAE-cellulose. The column (27.0 cm × 2.0 cm, diameter) was loaded with 22 100 units of nuclease. A 4.0-ml fraction was collected every 15 min. ●—●, nuclease activity; ○—○, alkaline phosphatase; △—△, protein.

TABLE III

INHIBITORS

The assays were carried out at pH 7.6, as described in *Assay of enzymes*, with reagents added as indicated. Enzyme solution was in 0.02 M Tris chloride, pH 7.6.

| *Addition* | *Concentration (M)* | *Inhibition (%)* | |
|---|---|---|---|
| | | *Nuclease I* | *Nuclease II* |
| EDTA | $10^{-3}$ | 8 | 6 |
| | $10^{-2}$ | 100 | 100 |
| | $4 \cdot 10^{-2}$ | 100 | 100 |
| $KH_2PO_4$ | $10^{-3}$ | 0 | 2.0 |
| | $10^{-2}$ | 11.0 | 11.5 |
| | $10^{-1}$ | 58.5 | 64.0 |
| $NaH_2PO_4$ | $10^{-3}$ | 2.5 | 2.0 |
| | $10^{-2}$ | 7.0 | 5.5 |
| | $10^{-1}$ | 35.0 | 47.0 |
| $ZnSO_4$ | $2 \cdot 10^{-4}$ | 54.5 | 39.5 |
| | $2 \cdot 10^{-3}$ | 91.3 | 96.2 |
| | $2 \cdot 10^{-2}$ | 100.0 | 100.0 |
| Spermidine | $10^{-4}$ | 0 | 0 |
| | $10^{-3}$ | 0 | 0 |
| | $10^{-2}$ | 26.5 | 18.5 |
| Polyvinylsulfate | 0.5 μg/ml | 89.0 | 91.2 |
| | 1.0 μg/ml | 100 | 100 |
| | 5.0 μg/ml | 100 | 100 |

4° and to many routine assay conditions but, in contrast to pancreatic ribonuclease, 3 min at pH 7 and 50°, and 60° result in a 55% and a 92% loss of activity, respectively.

*Activators and inhibitors:* Inhibition of the enzymic activity was measured by adding various agents as reported in Table III. Nuclease I and II were strongly inhibited by EDTA, zinc and polyvinylsulfate, and activated by $Mg^{2+}$. Optimum $Mg^{2+}$ concentration is dependent on the substrate used and is 0.01 M for the yeast RNA.

When $Mg^{2+}$ was added to the EDTA-inhibited nuclease, the activity was recovered to the original level (Table IV). From this result, it seems that both nuclease I and II require $Mg^{2+}$.

TABLE IV

REVERSIBILITY OF INHIBITION BY EDTA

The conditions were as described for Table III.

| *Addition* | *Concentration (M)* | *% Activity* | |
|---|---|---|---|
| | | *Nuclease I* | *Nuclease II* |
| None | — | 100 | 100 |
| EDTA | $10^{-2}$ | 0 | 0 |
| $MgCl_2$ ($+10^{-2}$ M EDTA) | $10^{-3}$ | 0 | 0 |
| | $5 \cdot 10^{-3}$ | 11.3 | 19.0 |
| | $10^{-2}$ | 93.0 | 84.0 |
| | $2 \cdot 10^{-2}$ | 86.5 | 76.5 |
| | $5 \cdot 10^{-2}$ | 34.0 | 35.0 |
| | $10^{-1}$ | 10.2 | 15.0 |

*Specificity of nuclease I and II:* The products of hydrolysis were shown to be 5′-phosphate terminated oligonucleotides as is described in detail in the accompanying paper. The principal products after extensive hydrolysis are dimers and trimers. No differences were detected between enzyme I and II.

In contrast to ribosomal nuclease, both enzyme fractions hydrolyze DNA to acid-soluble material. Enzyme assays with *p*-nitrophenyl phosphate and bis(*p*-nitrophenyl) phosphate as substrate detected neither phosphomonoesterase nor non-specific phosphodiesterase activity. Details of the base and conformation specificity are given in the accompanying paper.

## DISCUSSION

Nucleases I and II from the supernatant fraction of yeast are clearly distinguishable from ribosomal bound nuclease activity. The products of the ribosomal and supernatant nucleases differ in that the former are believed to be 3′-phosphate, mononucleotides[5,6] while the products of the supernatant nucleases I and II are 5′-phosphate terminated oligonucleotides, as shown in the accompanying paper. The enzymes are also clearly distinguishable by their pH optima and $Mg^{2+}$ requirement, by the effect of urea and EDTA upon them, and by the changes in apparent pH optima and EDTA sensitivity with changing growth phase.

A further distinguishing characteristic between the ribosomal and supernatant nucleases is that DNA (in particular when it is in a single-stranded state) is an excellent substrate for supernatant nucleases but is resistant to the ribosomal nucleases studied here. A description of the deoxyribonuclease activity will be found in the accompanying paper.

The several properties and the mode of hydrolysis of both nuclease I and II are quite the same. The yeast used here is a hybrid yeast; however, studies with the parent strains revealed that both had the same nuclease activities as judged by chromatographic patterns. OKAZAKI AND KORNBERG[14], studying DNA polymerase, demonstrated that similar chromatograms were the result of bound nucleic acid. Other suggestions have been offered by workers to explain similar behavior on DEAE-cellulose chromatography.

Changes in the activity of ribonuclease have been noted by DANNER AND MORGAN[6]. They report that ribosomal ribonuclease activity in yeast increased during cell growth, while ribonuclease activity in the soluble fraction did not change with time. In the present study, the authors found that the specific activity of ribosomal nuclease and soluble nuclease slightly increased (note the levels of protein used in Figs. 1 and 2) during the growth and the properties of ribosomal nuclease did not change. However, the nucleases in the supernatant had a higher pH optimum and greater sensitivity to EDTA in the logarithmic than in the stationary phase. It is interesting to speculate that these changes may have a functional relationship to control of protein and nucleic acid metabolism in the various growth stages of yeast.

The significance of the nuclease in the supernatant of yeast is not known. SPAHR AND SCHLESSINGER[15] indicated that a phosphodiesterase from *E. coli* supernatant solution, an exonuclease activated by $K^+$ and $Mg^{2+}$ (or $Mn^{2+}$), is responsible for the degradation of mRNA. The yeast supernatant nuclease is activated by $Mg^{2+}$ but not by potassium and produces primarily di- and trinucleotides, both of which are useless for the pool of nucleoside 5'-phosphates and would require further degradation before reuse.

ACKNOWLEDGEMENTS

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