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An Endonuclease from *Caenorhabditis elegans:* Partial Purification and Characterization

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A deoxyribonuclease was partially purified from the free-living nematode Caenorhabditis elegans. *The DNase functioned as an endonuclease and introduced both single-strand nicks and double-strand breaks into DNA. The enzyme hydrolyzed double-stranded DNA seven times more rapidly than single-stranded DNA. DNase activity was not affected by the addition of divalent cations below 1 mM but was inhibited at higher ionic concentrations. In addition, the enzyme was not inhibited in the presence of 10 mM EDTA. The enzyme was inhibited by salt concentrations greater than 20 mM. Three independent mutations in the* nuc-1 *gene were shown to reduce nuclease activity to less than 1% of that seen in wild-type organisms.*

KEY WORDS: deoxyribonuclease; *Caenorhabditis elegans;* acid DNase.

INTRODUCTION

The nematode *Caenorhabditis elegans* is currently the subject of much research, having been recognized as a model system for the study of the genetics, development, and biochemistry of a metazoan (for reviews, see Marx, 1984; Lewin, 1984a, b; Anderson and Kimble, 1987). *C. elegans* has a simple anatomy, consisting of an outer body wall and musculature that

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enclose the reproductive, nervous, and digestive systems. It reproduces largely as a self-fertilizing hermaphrodite, with each adult producing approximately 350 progeny. Large quantites of nematodes can be generated on agar-based casserole dishes that are layered with *Escherichia coli,* a food source. Eggs laid on the agar hatch and develop to adulthood in 3.5 days at 20°C. The genetics of *C. elegans* have been studied extensively. Thousands of mutants have been isolated and mapped to define over 500 genes (Edgley and Riddle, 1987). *C. elegans* has been employed to study cellular development, and the complete cell lineage has been traced such that the ancestry of each somatic cell in the adult is known (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979; Sulston *et al.,* 1983). The wealth of information concerning the biology of *C. elegans,* coupled with its ease of maintenance, renders it an ideal organism for a wide range of studies.

We have partially purified and characterized a deoxyribonuclease from *C. elegans.* The DNase isolated is likely encoded by the *nuc-1* gene (Sulston, 1976). Animals bearing a mutation in this gene are visibly indistinguishable from the wild type but lack a considerable amount of the enzyme activity seen in wild-type animals (Sulston, 1976; W. Wood, personal communication). Mutations in the *nuc-1* gene have been induced by a number of individuals (Sulston, 1976; H. R. Horvitz, personal communication). These aided in determining the function and clarifying the parameters of the *nuc-1* gene product.

MATERIALS AND METHODS

Animals. The wild type (N2) and a strain bearing the *e1392* mutation in *nuc-1* were obtained from the *Caenorhabditis* Genetics Center at the University of Missouri. Two additional *nuc-1* mutants, *nuc-1(n334)* and *nuc-1(n887),* were provided by H. R. Horvitz. Populations of *C. elegans* were grown on 21 × 21-cm casserole dishes containing NG agar (Brenner, 1974) layered with 15 ml of a mixture containing 0.5 g Bacto-Tryptone and 0.4 g NaCl in 50 ml water, a hen's egg, and 2 ml *E. coli* OP50 (Klass and Hirsh, 1981). The casserole dish was incubated at 37°C until the solution had dried onto the agar. Dishes were incubated with 2 to 3 ml of worms at 20°C for 3 to 4 days or until the majority of the population was adults. The casserole dish was washed with 1 liter of phosphate-buffered saline (PBS), *p*H 7.0, and the worms were allowed to settle out. This step was repeated two additional times. Animals were frozen at −80 or −196°C.

Nucleic Acids. ^{3}H-Labeled *Escherichia coli* DNA was prepared as described by Smith (1967). The isolated DNA was resuspended in 100 mM Tris, pH 8.0, 1 mM EDTA. When required, DNA was heat denatured for 3 min in a boiling water bath. For experiments in which reaction products were

analyzed by alkaline or neutral gel electrophoresis, DNA was extracted as described by Smith (1967) from an overnight culture of unlabeled *E. coli* W3110. Following ethanol precipitation, the pellets were air dried and stored at 4°C until resuspension in 500 μl 40 mM potassium phosphate buffer, *p*H 4.5.

E. coli [^{3}H]DNA was labeled with ^{32}P-nucleotides at its 3′ termini by first removing nucleotides from the 3′ ends and then carrying out repair synthesis in the presence of [α^{32}P]TTP (Hori *et al.*, 1983). Nucleotides were removed from the 3′ termini in a reaction mixture (0.5 ml) that contained 480 μl of 20 mM Tris–HCl, *p*H 8.0, 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 72 nmol of *E. coli* [^{3}H]DNA, and 300 units of *E. coli* exonuclease III. After 20 min at 37°C, 11% of the *E. coli* [^{3}H]DNA was rendered acid soluble. Following ethanol precipitation, the pellet was air dried and resuspended in 500 μl 20 mM Tris–HCl, *p*H 8.0, 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol. DNA was repaired by incubation for 60 min at 16°C in a reaction mixture that contained 1 mmol each of dCTP, dATP, and dGTP, 2 μCi of [α^{32}P]TTP, and 286 units of DNA polymerase I. The DNA was ethanol precipitated, washed twice with 70% ethanol, resuspended in 120 μl 100 mM Tris, *p*H 8.0, 1 mM EDTA, and stored at 4°C.

Plasmid pBR322 was isolated using the alkali lysis procedure described by Maniatis *et al.* (1982), resuspended in 10 mM Tris, *p*H 8.0, 1 mM EDTA, and stored at 4°C.

The concentration of DNA obtained from DNA extractions was determined using a fluorescent assay described by Labarca and Paigen (1980) with some modifications. DNA was added to a reaction mixture containing 3.4 ml of 50 mM Tris, *p*H 7.5, 10 mM $CaCl_2$, 10 mM $MgSO_4$, Hoechst 33258, added to a final concentration of 1 μg/ml, and distilled water, added to a final volume of 5 ml. Fluorescence was determined with a G K Turner Associates Model 111 fluorometer with the range selector set at 10×, a Turner 7-60 366-nm filter for the excitation light, a Turner 460-nm sharp cut filter, and a Turner 10% neutral density filter for the cutoff light. The DNA was diluted as needed with 40 mM potassium phosphate buffer, *p*H 4.5, and frozen in 1-ml aliquotes at −80°C. Once thawed, the DNA was stored at 4°C.

Reagents. Lysozyme was obtained from ICN Nutritional Biochemicals, Cleveland, Ohio. Exonuclease III was obtained from Pharmacia, Piscataway, N.J. DNA polymerase I was obtained from P. L. Biochemicals, Milwaukee, Wis. Restriction enzyme *Eco*RI, bovine serum albumin, cytochrome *c*, ovalbumin, Type VI agarose, phosphocellulose, bisbenzimide (Hoechst 33258), brilliant blue G (Coomassie blue), ethidium bromide, dATP, dGTP, dCTP, salmon sperm DNA, and RNase A were obtained from Sigma Chemical Company, St. Louis, Mo. [α^{32}P] Thymidine triphosphate (sp act, 25 Ci/mmol) and [*methyl*-^{3}H] thymidine (sp act, 20 Ci/mmol) were obtained from ICN Radiochemicals, Irvine, Calf.

Purification of Deoxyribonuclease. To prepare the N2 crude extract, animals (100 ml) were thawed at 4°C over a 3-day period, added to an equal volume of 40 mM sodium phosphate, *p*H 6.5, 1 mM EDTA, and sonicated on ice for 40 cycles using a Heat Systems Ultrasonics Inc. sonicator microtip on setting 7. One sonication cycle consisted of 15-sec pulse followed by a 45-sec cool-down period. The crude extract was observed with a dissecting microscope to assure that the carcasses had been disrupted and spun in a microfuge for 2 min. A second centrifugation was completed in a SW 50.1 rotor at 192,000 *g* for 2 hr at 4°C, and the supernatant brought to 40% saturation with solid ammonium sulfate, added over a 30-min period with constant stirring on ice. After an additional 60 min of stirring, the supernatant was centrifuged at 12,000 *g* in a SS34 rotor using a Sorvall RC2-B centrifuge for 30 min at 4°C. Precipitated material that was suspended throughout the tube was removed by passing the supernatant through a 20-μm nitex membrane filter (Tetko, Inc.), followed by a second filtration with a 5-μm cellulose nitrate membrane filter (Whatman). After saturation to 60% with ammonium sulfate and centrifugation as previously described, the resulting pellet was resuspended in 20 ml of 40 mM potassium phosphate buffer, *p*H 6.5 (column buffer), and dialyzed at 4°C against 1 liter of the same buffer with three changes (Fraction I). Fraction I was stored at 4°C.

Fraction I, diluted fourfold with column buffer, was applied at a flow rate of 0.5 ml/min to a phosphocellulose column (28 cm × 0.9 cm^2) previously equilibrated with column buffer. The column was washed with 100 ml column buffer and three 15-ml fractions were collected. Fractions were assayed for protein and DNase activity and stored at 4°C. The DNase was eluted from the column with a step gradient beginning at 100 mM NaCl, with increasing 50 mM increments to a final concentration of 500 mM NaCl. Sixteen 10-ml fractions were collected. Activity consistently eluted between 200 and 300 mM NaCl. All fractions were dialyzed against 1 liter column buffer and assayed for protein and DNase activity. Fractions that showed greater than 20% release of acid-soluble nucleotides in 30 min were pooled and stored at 4°C.

Protein concentrations of the various purification fractions were determined with a Bradford protein assay (Bradford, 1976).

Crude extract of three *nuc-1* strains as well as N2 were prepared by adding 3 ml frozen worms to 3 ml 40 mM phosphate buffer, pH 6.5, and sonicating as previously described. All extracts were spun in a microfuge for 2 min and the supernatant was assayed for nuclease activity.

DNase Assay. The assay measured the release of acid-soluble nucleotides from ^{3}H- or ^{32}P-labeled *E. coli* DNA. The standard assay mixture (0.5 ml) contained 4 nmol of *E. coli* [^{3}H]DNA, 40 mM potassium phosphate buffer, *p*H 4.5, and typically 10 μl of the enzyme preparation and was incubated at 37°C. Reactions were monitered by removing 100 μl at 0, 15, and

30 min. Reactions were stopped by the addition of 5 μl of a 5 μg/ml salmon sperm DNA solution and trichloroacetic acid to a final concentration of 10% and placed on ice for 15 min. Acid-soluble material was recovered by centrifugation in an Eppendorf microfuge for 15 min at 4°C. Radioactivity present in the supernatant was determined by counting in a Beckman Model LS 5801 scintillation counter. One unit of activity was defined as that amount causing the production of 1 nmol of acid-soluble nucleotides in 30 min. The assay of nuclease activity was linear to 20–30% release of acid-soluble material. Values of 30% or below were used to calculate units of activity.

Gel Electrophoresis. For experiments involving plasmid pBR322, reaction mixtures contained 20 μl of 40 mM potassium phosphate buffer, *p*H 4.5, 5 μl of resuspended pBR322 (15μg/ml), and 5 μl of the appropriate enzyme dilution and were incubated for 1–30 min at 37°C. Reactions were terminated by removing 15 μl to a second microfuge tube and adding 5 μl neutral tracking dye (5 mg bromophenol blue in 5 ml water and 5 ml glycerol). In addition, 5 μl of resuspended pBR322 (15 μg/ml) was diluted with 100 mM NaCl, 50 mM Tris–HCl, *p*H 7.5, 10 mM $MgCl_2$, 1 mM dithiothreitol and digested with 1 unit *Eco*RI for 30 min at 37°C. The majority (15 μl) of each sample was loaded onto a 0.8% agarose gel (Type VI agarose suspended in 80 mM Tris, *p*H 8.0, 2 mM EDTA, and 15 μl/ml 85% phosphoric acid) and electrophoresed at 60 V for 4 hr. The gel was stained for 1 hr in 1 mM $MgSO_4$ containing 1 μg/ml ethidium bromide and destained for 10 min in 1 mM $MgSO_4$. The gel was observed using a Chromato-Vue transilluminator (maximum emission, 300 nm) and photographed with Kodak Type 55 film. The negatives were scanned with a Joyce–Lobel densitometer.

For experiments utilizing *E. coli* DNA, RNA was first degraded by incubating for 15 min at 37°C with 40 μg/ml RNase A. Unlabeled *E. coli* W3110 DNA and *E. coli* [^{3}H]DNA were diluted in 40 mM potassium phosphate buffer, *p*H 4.5, to 65 and 4 nmol, respectively. Reaction mixtures contained 90 μl of the diluted DNA solution and 10 μl of the appropriate dilution of the phosphocellulose fraction. These were reacted for 15 min at 37°C. Reactions were stopped by removing 25 μl to each of two microfuge vials and adding 10 μl alkaline tracking dye (0.6 mg/ml bromocresol green in 0.3 N NaOH that is 40% sucrose) to one and 10 μl neutral tracking dye to the second. An additional 10 μl was removed, and the total radioactivity determined in a scintillation counter. TCA was added to the remaining reaction mixture to a final concentration of 10%, the tube spun 15 min in a microfuge, and 25 μl of the supernatant counted in a Beckman scintillation counter. The majority (25 μl) of the alkaline reaction mixtures was loaded on a 0.35% agarose gel (Type VI agarose in 30 mM NaOH, 2 mM EDTA), while 25 μl of each neutral sample was run on an 0.8% agarose gel (Type VI agarose suspended in 80 mM Tris, *p*H 8.0, 2 mM EDTA, and 15μl/ml 85% phosphoric

acid). Gels were electrophoresed at 12 V for 16 hr, stained, destained, and photographed, and the negatives scanned as described above. Samples were assayed for the amount of acid-soluble nucleotides released as described for the DNase assay.

RESULTS

Incubation of both the wild-type (N2) crude extract and the phosphocellulose fraction of *C. elegans* with radioactively labeled *E. coli* DNA resulted in the release of 20–30% acid-soluble material in 30 min (Fig. 1). Similarly prepared

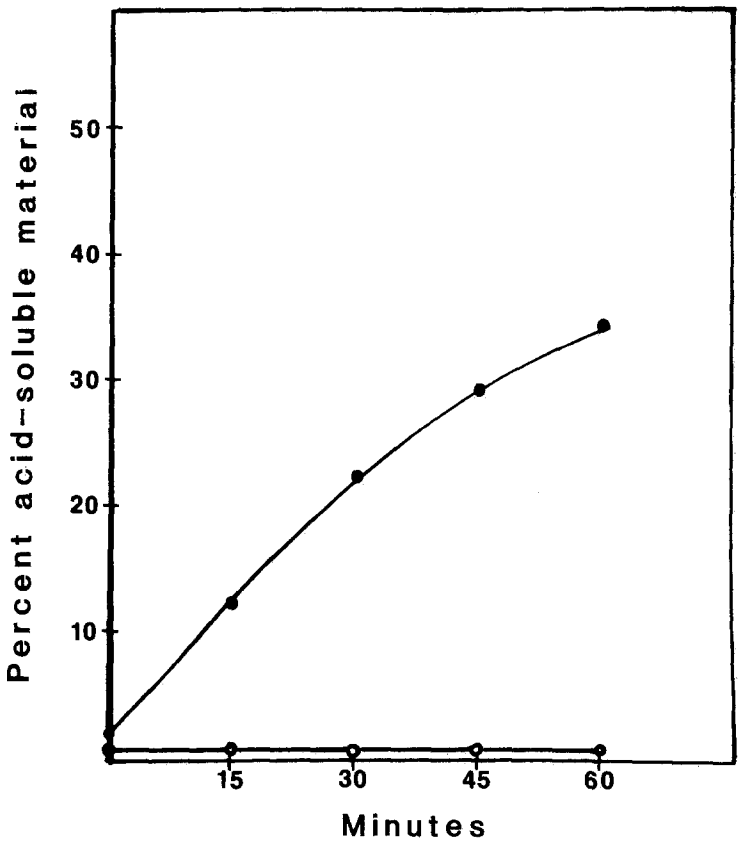

Fig. 1. Degradation of *E. coli* [^{3}H]DNA in the presence of N2 (●) and *nuc-1(e1392)* (○) crude extracts. Crude extracts were diluted to 2 μg/ml and 10 μl of each extract was assayed in each case. Activity was determined by reacting N2 and *nuc-1* crude extracts with 4 nmol DNA and measuring the release of acid-soluble nucleotides as described under Materials and Methods. Crude extracts of two additional alleles of *nuc-1*, (*n334*) and (*n887*), also released less than 1% acid-soluble material.

extracts of three strains bearing different mutations in the *nuc-1* gene were less than 1% as active as the N2 crude extract (Fig. 1); therefore, the observed activity was attributed to the *nuc-1* gene product. Unless otherwise noted, enzyme activity was measured by its ability to render high molecular weight DNA acid soluble.

Purification of *C. elegans* DNase

Partial purification of the DNase from *C. elegans* was achieved using ammonium sulfate precipitation and phosphocellulose chromatography and resulted in a 30-fold purification over the starting crude extract. Attempts at further purification with additional columns resulted in an unstable protein. Passing the active phosphocellulose fraction through a Sephadex column resulted in the loss of a majority of the enzyme activity within 12 hr when stored at 4°C. Conversely, the phosphocellulose fraction was stable for at least

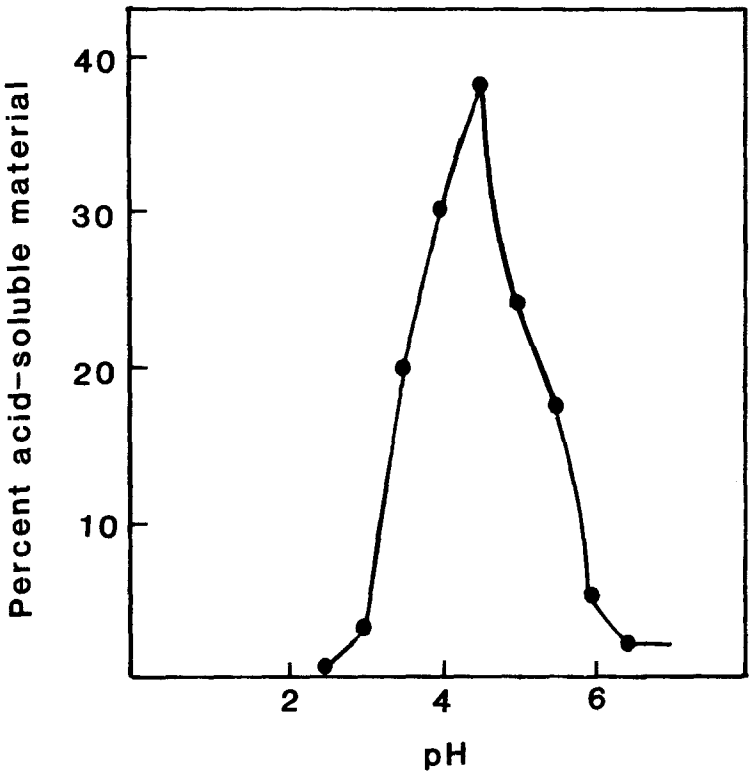

Fig. 2. Effect of *p*H on nuclease activity. Nuclease activity was determined by measuring the release of acid-soluble nucleotides as described under Materials and Methods. The *p*H of reaction mixtures ranged from 2.5 to 6.5.

6 months at 4°C in 40 mM potassium phosphate buffer, *p*H 6.5. It could also be stored at −80°C for 10 months in the presence or absence of 50% glycerol with no detectable loss in activity. The phosphocellulose fraction does not represent a pure sample; there were certainly other proteins and possibly other nucleases present in the fraction. However, the standard assay was conducted at *p*H 4.5 and in the presence of EDTA, conditions that are inhibitory to many enzymes but optimal for this DNase. Mutations in *nuc-1* virtually abolish nuclease activity (Fig. 1). Three *nuc-1* mutants were assayed at *p*H values ranging from 4.5 to 6.5 and in the presence and absence of EDTA. Activity was not detected in any of these strains under any of these conditions. The active phosphocellulose fraction was used to characterize the properties of the enzyme.

Properties of the Nuclease

The enzyme had a *p*H optimum of 4.5 and was approximately seven times as active at *p*H 4.5 as compared with *p*H 6 (Fig. 2). Native DNA was hydrolyzed

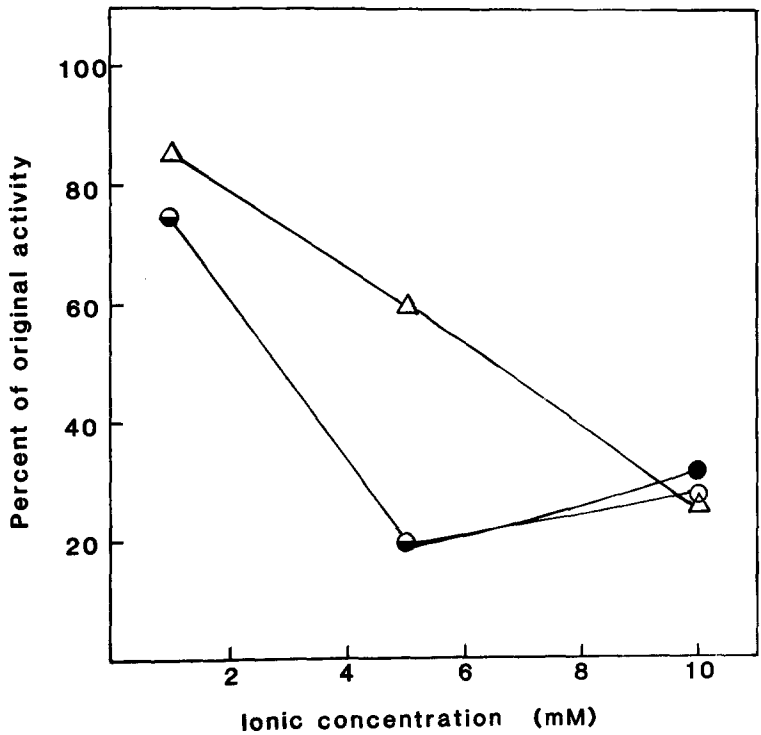

Fig. 3. Effect of divalent cations on nuclease activity. Nuclease activity was assayed as described under Materials and Methods except that increasing amounts of $MgCl_2$ (○), $MnCl_2$ (●), and $CaCl_2$ (△) were added to the reaction mixture.

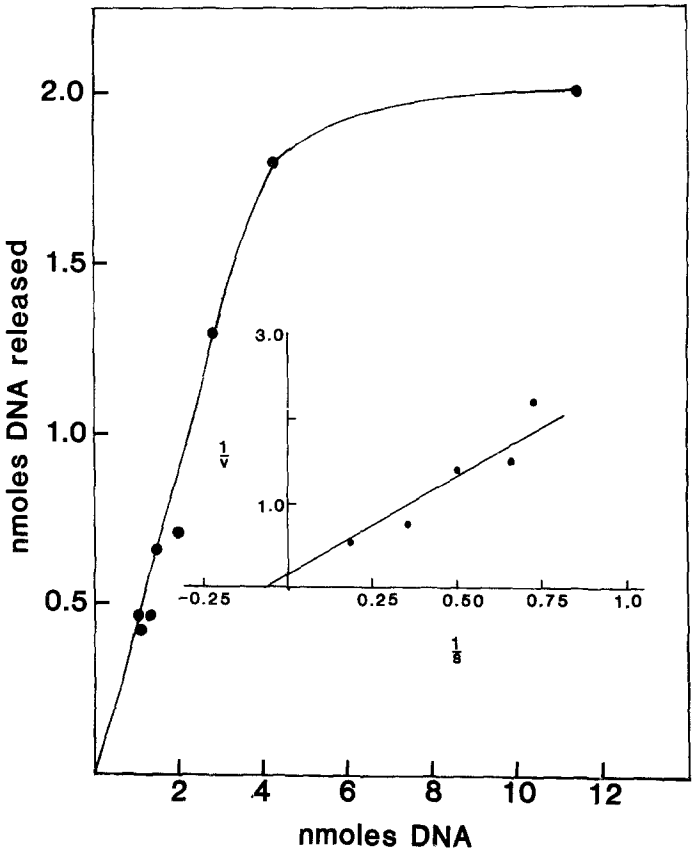

Fig. 4. Effect of DNA concentration on reaction rate. N2 phosphocellulose fraction was added to reaction mixtures containing from 1 to 12 nmol DNA. Reactions were terminated at 15 and 30 min, and the amount of acid-soluble nucleotide was determined as described under Materials and Methods. The inset is a Lineweaver–Burke plot of the data.

seven times more rapidly than heat-denatured DNA. Enzyme activity was not significantly inhibited by magnesium, manganese, and calcium concentrations below 1 mM, while concentrations of 10 mM of these divalent cations reduced activity 70–75% (Fig. 3). Enzyme activity was not inhibited by 10 mM EDTA but was inhibited by salt. A 50% reduction in activity was seen at 20 mM NaCl, while 10% of the original activity remained at concentrations of 100 mM NaCl.

The rate of hydrolysis of DNA was proportional to the amount of DNA added up to 4 nmol DNA (Fig. 4). The K_m was calculated to be 1.7×10^{-5} M and the V_{max} was determined to be 6.7 nmol DNA released in 30 min. Accurate measurements of reaction rate were not obtained at substrate concentrations greater than 12 nmol DNA. Activity was not detected at

higher substrate concentations because the acid-soluble release assay measures enzyme activity by the release of acid-soluble nucleotides and cannot detect nicking activity. Experiments presented subsequently indicate that the enzyme acts by first breaking high molecular weight DNA into fragments which are then further degraded to small acid-soluble nucleotides. With a large amount of substrate, the enzyme cannot produce acid-soluble nucleotides in the given time.

The reaction rate was linear with increasing enzyme concentrations to 10 μl of enzyme added. Higher concentrations of enzyme did not serve to increase the rate of the reaction.

Three different experiments were conducted to determine the mode of hydrolysis of the DNase. The first showed that the enzyme degraded

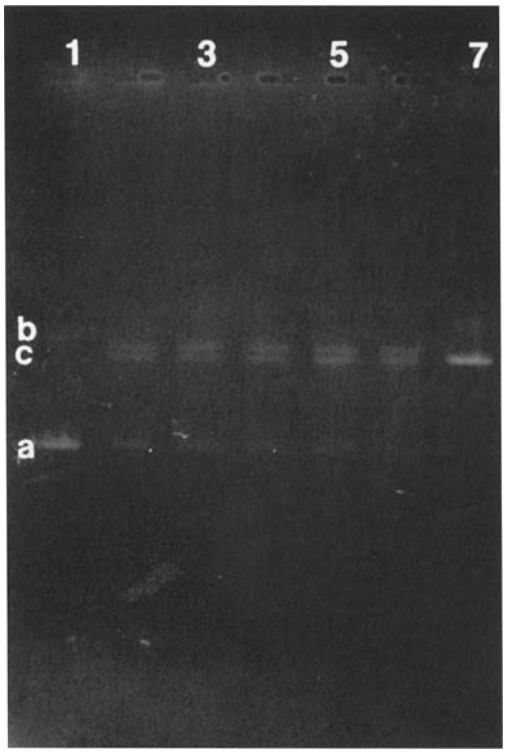

Fig. 5. Neutral gel showing the reaction of plasmid pBR322 with the enzyme. Bands representing the three conformations of DNA are (a) closed-circular DNA, (b) open-circular DNA, and (c) linear DNA. Lanes 1–6: Time points, beginning with lane 1, are 0, 1, 5, 10, 20, and 30 min. Lane 7: 30-min *Eco*Rl digest of pBR322.

closed-circular plasmid pBR322, indicating endonuclease capabilities. In the second, incubation of the enzyme in the presence of end-labeled DNA indicated either that the enzyme hydrolyzed DNA in both the 3′–5′ and the 5′–3′ directions or that the DNase was not an exonuclease. Finally, the reaction products were sized on both alkaline and neutral gels, which gave further evidence that the enzyme was an endonuclease. All results indicate that the enzyme functioned as an endonuclease, capable of making both single- and double-strand breaks in the DNA.

In the first set of experiments, the enzyme was reacted with closed-circular plasmid pBR322 and the reaction products were separated by electrophoresis through neutral agarose (Fig. 5). Densitometric scans showed that approximately 85% of the plasmid preparation was closed-circular. During incubation with the enzyme, this peak decreased in amount, while the open-circular and linear peaks increased. Greater than 99% of the closed-circular DNA had disappeared in 30 min. The intensities of the open-circular and linear bands were roughly equal, indicating that single- and double-strand breaks were induced at approximately equal rates.

Incubation of the DNase with [^{3}H–3′-^{32}P]DNA for 30 min resulted in the

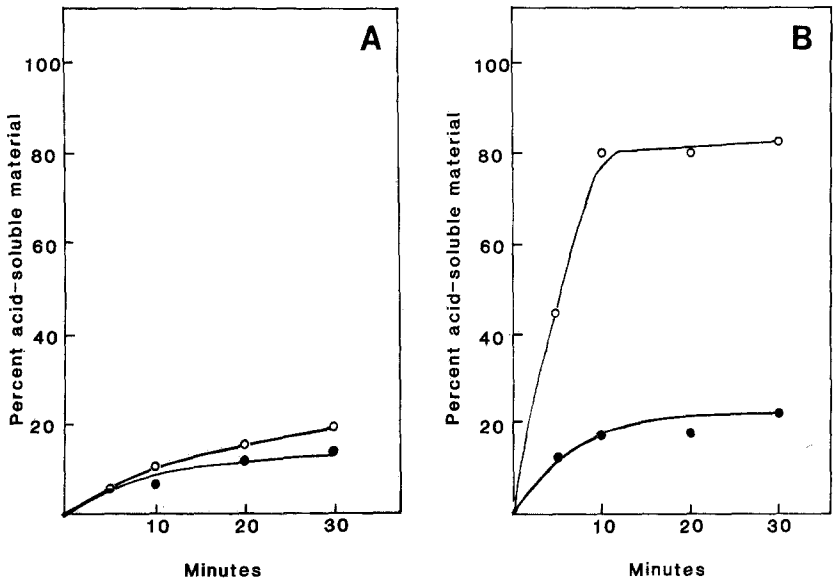

Fig. 6. Hydrolysis of ^{3}H-labeled (●) *E. coli* DNA labeled at its 3′ termini with ^{32}P (○). The dual-labeled DNA was incubated with the active N2 phosphocellulose fraction or with *E. coli* exonuclease III, a 3′-specific exonuclease. Activity was determined by the amount of each label released as acid-soluble material as described under Materials and Methods. (A) Digestion with the *C. elegans* phosphocellulose fraction. (B) Digestion with *E. coli* exonuclease III.

release of 14% of the ^{3}H label and 19% of the ^{32}P label as acid-soluble material (Fig. 6). When [^{3}H–3′-^{32}P]DNA was reacted with *E. coli* exonuclease III, a 3′-specific exonuclease, 23% of the ^{3}H label and 82% of the ^{32}P label were rendered acid soluble (Fig. 6). DNA that had been labeled at its 5′ ends had rates of release similar to those obtained with 3′ end-labeled DNA (data not shown). Thus, the enzyme either lacked an exonuclease activity or possessed both 5′–3′ and 3′–5′ exonuclease activities.

These possibilites were distinguished by analyzing digestion products from the same set of reactions using the acid-soluble release assay as well as alkaline and neutral gel electrophoresis. The substrate concentration was increased 15-fold in order to visualize DNA in the gels. Under these

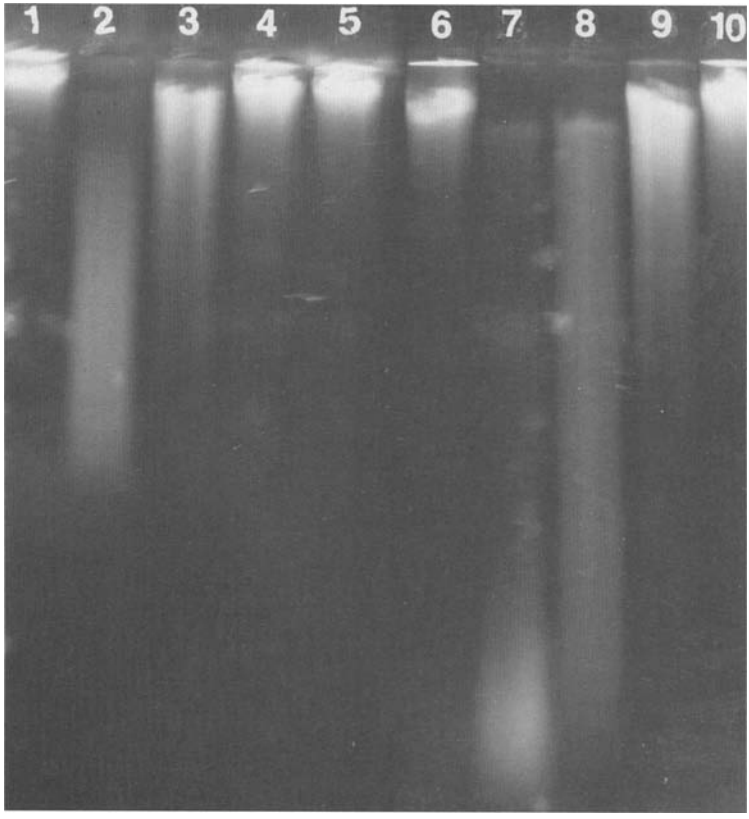

Fig. 7. Photograph of neutral (Lanes 1–5) and alkaline (Lanes 6–10) gels showing the effects of N2 phosphocellulose fraction on high molecular weight *E. coli* DNA. The *E. coli* DNA was reacted for 30 min with no enzyme (Lanes 1 and 6), undiluted enzyme (Lanes 2 and 7), a 1:5 dilution of enzyme (Lanes 3 and 8), a 1:25 dilution of enzyme (Lanes 4 and 9), or a 1:125 dilution of enzyme (Lanes 5 and 10).

conditions, less than 5% acid-soluble material was released in a 30-min incubation period. Alkaline gel electrophoresis indicated that, despite this apparent lack of activity, incubation of the DNase with *E. coli* DNA resulted in products migrating near the bottom of the gel, indicating extensive degradation (Fig. 7). The extract could be diluted fivefold and still produce substantial albeit lesser reductions in molecular weights (Fig. 7). These results are consistent with an endonuclease, as an exonuclease would be expected to release acid-soluble material during this time.

The data using pBR322 as a substrate suggest that the nuclease produced both single-strand nicks and double-strand breaks in the DNA. Neutral gels, run under nondenaturing conditions, were used to confirm these results. Double-strand breaks fragment the DNA, which then appears on a neutral gel as lower molecular weight DNA. Nicked DNA will remain high molecular weight. Results obtained from the neutral gel (Fig. 7) show that the molecular weight of the DNA was reduced substantially after reaction with the enzyme. Therefore, these data collaborate the results using pBR322 that indicate that the enzyme can make both single- and double-strand breaks in the DNA.

The enzyme released less than 5% acid-soluble material in this experiment, while releasing as much as 30% acid-soluble material using the standard assay. This discrepancy can be explained by the fact that the standard assay reaction mixture contained 4 nmol DNA, which the DNase fragmented and then degraded to acid-soluble nucleotides within 15 min. The reactions to be analyzed on neutral and alkaline gels contained 15-fold more DNA, and as was discussed previously, the enzyme was unable to degrade the DNA to fragments of acid-soluble size in the given time.

DISCUSSION

A DNase from *C. elegans* was partially purified and some of its properties were characterized. The enzyme functioned as an endonuclease with an optimum *p*H of 4.5. The enzyme degraded native DNA seven times more rapidly than denatured DNA. It did not require divalent cations, functioned in the presence of EDTA, and was inhibited by NaCl. This particular enzyme represents a major DNase present in *C. elegans*. It is likely encoded by the *nuc-1* gene since animals bearing mutations in this gene lack the enzyme activity. However, it is possible that the *nuc-1* gene product instead acts to regulate expression of the structural gene for the enzyme.

The DNase functioned as an endonuclease as shown by the production of open-circular and linear DNA when incubated with closed-circular DNA. The presence of both products in approximately equal amounts following enzymatic degradation suggests that the DNase produced both single- and

double-strand breaks in the DNA. Results of alkaline gels also indicate that the enzyme was an endonuclease. Low molecular weight DNA was obtained after incubation with the enzyme, but reaction mixtures prepared for alkaline gels did not contain acid-soluble nucleotides, indicating little, if any, exonucleolytic activity. The DNase apparently functioned by cutting the DNA at a number of sites, resulting in fragments of DNA that were then degraded further to products of acid-soluble size.

The DNase isolated here is very similar to the acid deoxyribonucleases, which are widely distributed in animal cells (Bernardi, 1971; Rasskazov *et al.*, 1986). The acid DNases are double-strand specific endonucleases that can introduce single- and double-strand scissions in DNA and are often inhibited by magnesium ions. They appear to be inhibited by high substrate concentrations when the rate of hydrolysis is determined with an acid-soluble release assay. These properties also describe the *nuc-1* gene product.

In addition, acid DNases are localized in lysosomes and function in the degradation of cellular DNA. There is the possibility that the *C. elegans* DNase is a lysosomal enzyme, as it is a hydrolytic enzyme that functions optimally in an acidic environment. Lysosomes and lysosomal enzymes are acidic in nature (Coffey and DeDuve, 1968), and lysosomal extracts have been shown to degrade nucleic acids (Arsenis *et al.*, 1970).

The availability of *nuc-1* mutants has made determination of the physiological role of the DNase possible. It does not play a requisite role in DNA replication, as *nuc-1* mutants show normal growth rates. The DNase is not essential for DNA repair, as *nuc-1* animals do not exhibit increased hypersensitivity to UV or X radiations (Hartman and Herman, 1982). Animals bearing the *nuc-1* mutation do not show rates of recombination that vary from those seen in wild-type animals (Edgley and Riddle, 1987), indicating that this DNase is not required for recombination. Rather, this nuclease functions primarily in the degradation of cellular DNA as well as scavenging nutritionally derived DNA from the gut. This is evidenced by the observation that *nuc-1* animals are unable to degrade DNA in cells that have undergone programmed cell deaths. During *C. elegans* development, cells follow distinct patterns of divisions, with most cells having specific fates in the adults (Sulston *et al.*, 1983). Some cells are destined to undergo cell deaths, a process in which the cell dies and is removed by phagocytosis. In *nuc-1* animals, the nucleoplasm of dead cells is removed, while the DNA is not degraded and can be detected by staining with Feulgen (Hedgecock *et al.*, 1983). The inability of *nuc-1* animals to degrade cellular DNA indicates that the *nuc-1* gene product functions in this capacity. In addition, Feulgen staining has shown DNA in the gut of *nuc-1* animals, indicating that nutritionally dervied DNA is not degraded (P. Babu, personal communication).

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