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Purification of an Endonuclease Present in *Chrysaora quinquecirrha* Venom (41077)

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Abstract. A DNase was isolated from the nematocyst venom of the sea nettle (*Chrysaora quinquecirrha*). This enzyme was purified 880-fold by sequential affinity chromatography. The enzyme had a pH optimum of 6.8, a molecular weight of 110,000 daltons, an isoelectric point of 6.9, and was active only on single-stranded DNA. The activity of the DNase could be inhibited by bivalent ions (Ca^{2+}, Mg^{2+}, and Zn^{2+}) but was stimulated by sodium chloride. The nettle DNase could incise superhelical DNA only in low saline concentrations. Nettle DNase lacked 3'- to 5'-exonucleolytic activity, and its endonucleolytic activity produces cleaved reaction products containing 5'-phosphoryl or 3'-hydroxyl end nucleotides.

A number of jellyfish venoms have been studied in detail. The pharmacological properties of these venoms include cardiac, dermonecrotic, hemolytic, and proteolytic activities (1). These toxicological effects are thought to be mediated by various polypeptides and enzymes present within the venoms. Proteolytic enzymes have been detected in three jellyfish venoms (*Physalia physalis, Chrysaora quinquecirrha,* and *Stomolophus mealeagris*) (1, 2). However, only *Chrysaora* and *Physalia* possess DNase.

The presence of different types of DNase in other animal venoms has been known for years (3–7). Pritchard *et al.* (8) found an endonuclease specific for single-stranded and superhelical DNA in the venom of a snake, *Crotalus adamanteas*. Slor, *et al.* (9) discovered several DNases within the oriental hornet venom (*Vespa orientalis*). Because of the deleterious nature of these enzymes, their ubiquitousness, and their possible role in the pathogenesis of a sting, the main DNase of sea nettle venom has been purified and investigated.

Material and Methods. Sea nettle medusae were collected in Meredith Creek, St. Margaret's, Maryland, in the summer of 1977. The fishing and mesenteric tentacles were removed manually and the separation of nematocysts from the tentacles was performed by homogenization and centrifugation as previously described (10, 11) to obtain an isolated nematocyst suspension.

Whatman DEAE-cellulose (DE-52) was used after precycling. Hexylamine Sepharose was prepared as described earlier (12) utilizing Sepharose 4B (Pharmacia Fine Chemical Co., Piscataway, N.J.). The cyanogen bromide activation of the Sepharose was done as described by March *et al.* (13). Bovine serum albumin, catalase, yeast alcohol dehydrogenase, and soybean yeast lipoxidase were purchased from Schwarz/Mann Company, Orangeburg, New Jersey: Pharmacia and Sigma Chemical Company, St. Louis, Missouri. Tritium-labeled ϕX 174 RF1[1] DNA prepared by the method of Feldberg and Grossman (14) had a specific activity of $2-3 \times 10^5$ cpm/μg. Tritium-labeled *Escherichia coli* DNA prepared according to the method of Mahler (15) had a specific activity of 1.2×10^4 cpm/μg.

Calf thymus DNA and transfer RNA were obtained from Sigma Chemical Company. λ phage DNA was purchased from Bethesda Biochemical Laboratory, Bethesda, Maryland. Venom phosphodiesterase was purchased from Worthington Biochemical Corporation, New Jersey. DNA polymerase 1, [^{3}H]dGTP, and [^{3}H]dTTP were generously provided by Dr. R. Padmanabhan of the University of Maryland School of Medicine.

[1] Abbreviations used: RF1, Replicative form 1; Snake venom phosphodiesterase (E.C. 3.1.4.1.); Bovine spleen phosphodiesterase (E.C. 3.1.4.18).

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DNase assays. Acid-soluble fraction assay. The assay was based on the conversion of thermally denatured or native [^{3}H]DNA, to acid-soluble products (16). The standard assay mixture of 0.2 ml contained 1.3 μg DNA in 10 m*M* Tris, pH 7.0, buffer with 100 m*M* NaCl. The test sample was added and the extent of solubilization determined when the reaction was stopped after a 30-min incubation at 37° by the addition of 0.1 ml of cold 20% trichloroacetic acid and 0.05 ml of calf thymus DNA solution (1 mg/ml). The assay was linear at this point. The sample was immersed in ice for 10 min then centrifuged at 7000 *g* for 15 min at 4°. The radioactivity present in 0.2 ml of supernatant was counted in 10 ml Biofluor scintillation counting fluid (New England Nuclear Co., Boston, Mass.). The supernatant fluid obtained from reagent control incubations contained 0.05% of the added radioactivity. One unit of DNase converted 0.3 μg of denatured *E. coli* DNA to an acid-soluble form, under the conditions described.

Incision assay. The incision assay was done according to the method of Braun and Grossman (17). A mixture containing [^{3}H]ϕX 174 RF1 DNA, 0.2 ml 10 m*M* Tris (pH 7.0) buffer with 10 m*M* NaCl, was incubated at 37° for 30 min. The nucleic acid was denatured by the addition of 2 ml 10 m*M* Tris (pH 12) buffer containing 100 m*M* sodium phosphate, 30 m*M* sodium chloride, and 25 m*M* EDTA. This mixture was later neutralized to pH 7.6 by adding 0.4 ml of 25 m*M* Tris/HCl (pH 4.0) solution. Under these conditions [^{3}H]ϕX RF1 DNA was conserved, but the incised DNA was converted to at least two elongated single-stranded pieces. The RF1 DNA passed through nitrocellulose membrane filters (B-6 filters, Schleicher and Schuell) (18), whereas, the single-stranded DNA was retained on the nitrocellulose filters. The filters were washed twice with 4 ml NaCl citrate solution (300 m*M* NaCl and 30 m*M* citrate), dried, and counted in 10 ml Instafluor counting fluid (Packard Instrument Co., Downers Grove, Ill.).

Sedimentation analysis. Sedimentation analysis of the reaction products were carried out in a Spinco ultracentrifuge with a SW-50.1 rotor. Linear 5–20% (w/v) sucrose gradients (4.5 ml) containing 0.1 *M* EDTA, 0.3 *M* NaOH, and 1.7 *M* NaCl were formed in cellulose nitrate tubes. Fractions (0.18 ml) were collected from the bottom. Radioisotope content was determined after addition of 10 ml of Biofluor.

Chromatographic analysis. E. coli [^{3}H]DNA was heat denatured and digested (37°, 10 m*M* Tris, 20 m*M* NaCl) with 25 units of DNase for 3 hr after which an additional 15 units was added and incubation continued for an additional hour. The reaction was stopped by submerging the tubes in 100° water. The DNA was fractionated on a DEAE-cullulose column (0.4 × 10 cm) which had been equilabrated with 10 m*M* Tris, 1 m*M* EDTA, 7 *M* urea, pH 7.6, and eluted with a 0–0.5 *M* NaCl gradient. The resulting nucleotides were compared with authentic samples (kindly supplied by Drs. L. Hamilton and D. Padmanabhan).

Protein determination. Protein was determined by the method of Lowry *et al.* (19) or Groves *et al.* (20).

Ultraviolet irradiation. Tritium-labeled ϕX 174 RF1 DNA in 1 m*M* Tris buffer (pH 7.3) containing 1 m*M* EDTA was irradiated in a 2-mm pathlength. Radiation was emitted primarily at 254 n*M* from a low-pressure mercury lamp (Sylvania). The amount of incident radiation at 2500 erg/mm^2 was determined by a Black Ray short-wave ultraviolet monitor (Ultra-Violet Products, San Gabriel, Calif.).

Molecular weight determination. The molecular weight was determined using a Biogel A 0.5 M (Bio Rad Laboratories, Richmond, Calif.) column (1.5 × 37.6 cm). The sample was 1 ml of the purified enzyme containing 0.25 mg of protein. The eluate was 250 m*M* potassium phosphate buffer, pH 7.0, containing 5% glycerol. The standards were bovine serum albumin, soybean lipoxidase, yeast alcohol dehydrogenase, and catalase. The standards were detected by 280 nm absorbance. The enzyme was analyzed by its action in releasing acid-soluble products from heat-denatured [^{3}H]ϕX 174 RF1 DNA, and by incision assay using native [^{3}H]ϕX 174 RF1 DNA.

DNA denaturation. All DNA denaturation was done by boiling the DNA solution for 10 min before immediate cooling.

Polymerase-catalyzed incorporation of nucleotides into λ phage DNA. This reaction was performed according to the method of Wu *et al.* (21) with slight modifications. Tritiated nucleotides ([^{3}H]dGTP and [^{3}H]dTTP) were employed. The reaction was carried out at 37° for 2 hr. The radioactive DNA was denatured by heat treatment as described above.

Isolation and purification of the endonuclease. Preparation of crude nematocyst extract. The isolated nematocyst suspension was stored at −70° until use at which time 10 g of this suspension was thawed and added to 10 ml Buffer A (10 m*M* Tris, pH 7.0, 2 m*M* 2-mercaptoethanol, 5% glycerol). The resultant suspension was sonicated four times for 30-sec periods with a Branson sonifier (Branson Instrument, Danbury, Conn.) with a 15-min cool-down period between sonications. All operations were performed at 0−4°. After sonication was complete, the tissue particles were removed by centrifugation at 4° for 60 min at 18,000*g*.

Forty milliliters of Buffer A containing 3.2% w/v streptomycin sulfate was slowly added under gentle stirring to the sonicated supernatant. After 1 hr the resultant precipitate was removed by centrifugation. The supernatant was dialyzed for 24 hr against Buffer X (10 m*M* potassium phosphate buffer, pH 7.3; 1 m*M* 2-mercaptoethanol; 5% glycerol).

DEAE-cellulose chromatography. A column of precycled Whatman DE-52 (1.6 × 2.5 cm) was prepared and equilibrated with 3 liters Buffer X. The dialyzed crude extract was loaded on the column with a flow rate of 60 ml/hr and washed with 250 ml Buffer X. The protein was eluted in 12-ml fractions with an 800-ml linear gradient of Buffer X containing 0−300 m*M* KCl. The column was then washed with 150 ml of Buffer X containing 0.5 *M* KCl.

Hexylamine Sepharose chromatography. A column of hexylamine Sepharose (0.8 × 4.5 cm) was equilibrated with 100 ml Buffer Y (1 m*M* sodium potassium phosphate, pH 6.9; 5% glycerol). Four milliliters of the sample to be introduced was dialyzed for 3 hr against 3 liters of Buffer Y before being applied to the column at a rate of 4 ml/hr.

The column was washed with 8 ml sodium potassium phosphate Buffer Y and eluted successively with 16-ml aliquots of the same solution containing progressively increasing buffer sodium chloride concentrations of 1, 10, 25, 50, and 75 m*M*. The eluate was collected in 4-ml fractions and tested for DNase activity. Active fractions were pooled, stored at −70°, and later concentrated by solvent absorption through dialysis tubing into dry Sephadex G-200 beads.

Disc gel electrophoresis. Disc electrophoresis was performed according to earlier techniques (22). The DNase purified by hexylamine Sepharose column chromatography was introduced on the gel in 0.1-ml samples (2 μg protein). After the electrophoresis the gel was cut to 2-mm disks and extracted overnight at 4° in 10 m*M* Tris buffer (pH 7.0) containing 5% glycerol and 100 m*M* NaCl. After 24 hr, the DNase activity was assayed by the release of acid soluble products from *E. coli* [^{3}H]DNA and by an incision assay, using [^{3}H]φX 174 RF1 DNA.

Purity of the DNase was assessed by gel electrophoresis of the hexylamine Sepharose-purified product. After staining the gel with Coomassie Blue, the purity was determined by microdensitometric scan (MK 11 C, Loeble Co. Ltd., Princway Team Valley, England).

Isoelectric focusing. Aliquots (0.05 ml) of test fractions eluted from the hexylamine Sepharose column were subjected to analytical thin-layer isoelectric focusing on an LKB (LKB Produkter, Bromma, Sweden) Ampholine PAG plate (pH 3.5 to 9.5) which had been focused for 2 hr. Three-millimeter strips were cut from the plate and extracted overnight at 4° in 10 m*M* Tris buffer (pH 7.0) containing 5% glycerol and 100 m*M* NaCl. After 24 hr the DNase activity was assayed as above.

Assay for 3'- to 5'-exonucleolytic activity. One microgram of heat-denatured λ phage DNA labeled with tritiated nucleo-

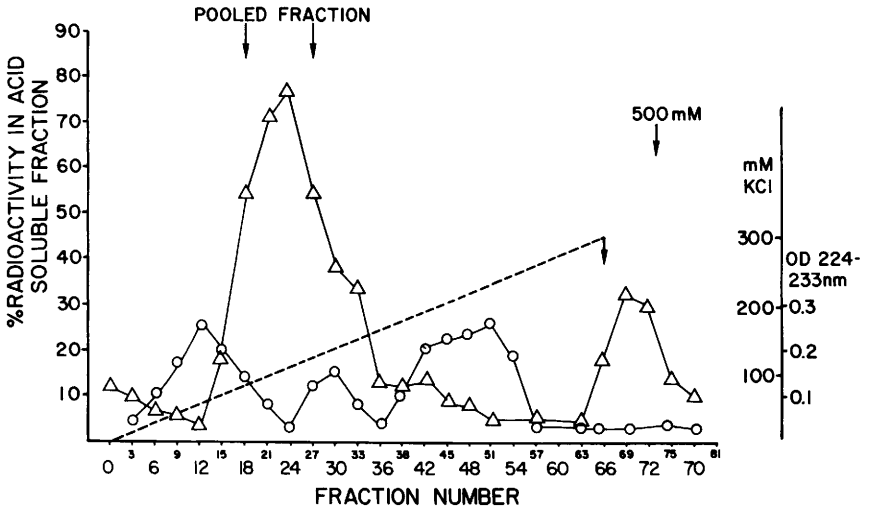

FIG. 1. Elution pattern of DNase activity from a DEAE-cellulose (DE-52) column (1.6 × 25 cm, KCl gradient). The crude venom after streptomycin sulfate treatment (80 ml) was applied to the column and eluted as described in the text. Protein concentration was determined by subtracting the optical absorption reading at 233 nm from that at 224 nm. △, DNase activity measured by the release of acid-soluble material. ○, Absorbance measured at 224–233 nm.

tides (1200 dpm/μg) placed on the 3′ end of the molecule was incubated at 37° in 10 m*M* Tris buffer (pH 7.0) containing 50 m*M* NaCl. Nettle endonuclease or snake phosphodiesterase (a control) was inoculated into the reaction mixture and the release of radioactive nucleotides was determined by the acid-soluble assay.

Results. DEAE-cellulose (DE-52) chromatography gave good resolution of the enzymatic activity (Fig. 1). Two active DNase peaks were detected in the 100 and 500 m*M* KCl eluates, respectively.

Fractions 18–27 containing the first and largest of these two peaks were pooled. Four milliliters of that pool was chromatographed on a hexylamine column and eluted with increasing concentrations of phosphate buffers. The most active fraction was eluted with 10 m*M* phosphate buffer (Fig. 2). The DNase activity was assayed by the release of acid-soluble products from heat-denatured *E. coli* [^{3}H]DNA and by incision assay using [^{3}H]ϕX 174 DNA. Both assays showed maximum activity in fraction 5. This combination of DEAE- and hexylamine Sepharose columns followed by concentration increased the specific activity of the enzyme 880-fold (Table I). Only one enzymatic peak was detected by gel electrophoresis of the active fractions eluted from the hexylamine column. The presence of the active enzyme within the gel was determined by extracting the gel sections and analyzing with the acid-soluble and the incision assays. Approximately 75–80% of the enzymatic activity (assayed as above) was lost in the gel electrophoresis procedure. The R_f of this single DNase recovered from the gel was 0.6 compared to bromphenol blue. Two bands were seen on staining with the main band at R_f 0.6. A microdensitometric scan indicated 85% of the total protein was in this band with the remaining protein appearing at R_f 0.7.

Isoelectric focusing. Isoelectric focusing of the DNase recovered from the combined affinity chromatography revealed that one peak of DNase activity occurred at p*I* 6.9 (Fig. 3). Only 25% of the DNase activity was recovered.

Molecular weight determination. The molecular weight of the active enzyme eluted from the hexylamine column was calculated to be 110,000 daltons when compared with known standards which were inoculated onto the same column and detected by 280 m*M* absorbance (Fig. 4).

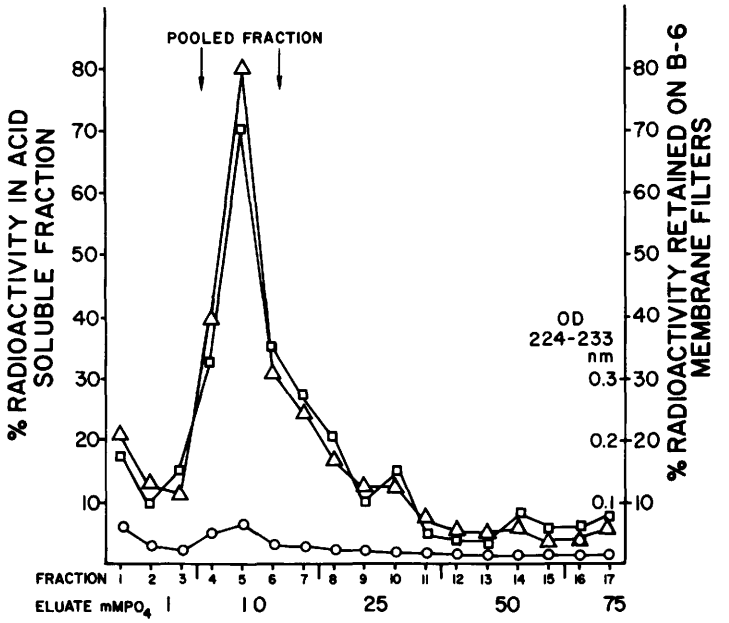

FIG. 2. Hexylamine Sepharose chromatography. Four milliliters from the pooled fraction after DE-52 chromatography was dialyzed and applied to hexylamine Sepharose column (0.8 × 4.5 cm). The column was eluted with increasing concentrations of potassium phosphate buffer. DNase activity was measured by two assays. (□), The release of acid-soluble material of *E. coli* heat-denatured DNA. 0.3 μg DNA, specific activity 10^4 dpm/μg. (△), Percentage radioactivity retained on B-6 membrane filter (0.1 μg φX RF1 DNA with specific activity 4×10^4 dpm/μg).

Stability-purified endonuclease. After sequential DEAE-cellulose and hexylamine Sepharose chromatography, the purified enzyme lost its activity in 10 days at 4° incubation even if placed in a 10 m*M* sodium potassium phosphate (pH 7.0) buffer containing 20% glycerol. It was stable, however, for at least 3 months at −70° under the same conditions.

A half-life of 40 and 30 min was observed

TABLE I. SUMMARY OF PURIFICATION OF NETTLE ENDONUCLEASE

Fraction	Procedure	Total protein (mg)	Total DNase Activity (U × 10^{-2})	Percentage yield	Specific activity (U/mg protein × 10^{-2})
I	Removal of nematocyst capsule	230	2360	100	10.2
II	Streptomycin treatment and dialysis	172	2240	95	13.0
III	DEAE-cellulose chromatography	3.5	1660	75	474
IV	Hexylamine Sepharose chromatography	0.020	44.9[a]	55[b]	2245
V	Concentration of Fraction IV by solvent absorption into dry Sephadex G-200 beads	0.005	44.9	55[b]	8980[c]

[a] Only 4 ml of Fraction III (2.2%) was chromatographed on hexylamine Sepharose.
[b] The yield was calculated assuming the entire amount of Fraction III was used.
[c] Calculated assuming total protein to be maximal.

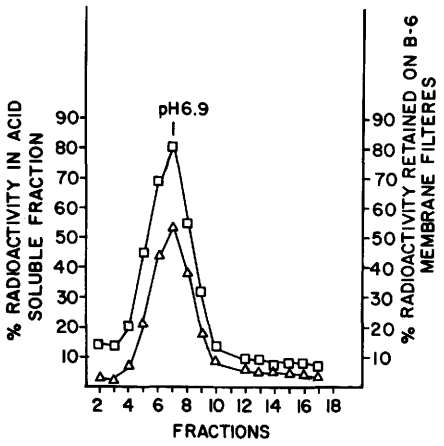

FIG. 3. Analytical isoelectric focusing of the DNase purified by DEAE- and hexylamine Sepharose chromatography. The sample was 0.1 mg; the activity was extracted from the electrophoresis gel sections as described in the text. Only 25% of the activity was recovered after the extraction from the gel. △, The release of acid soluble material of *E. coli* heat-denatured DNA (0.3 μg DNA; specific activity 10^4 dpm/μg). □, The φX incision assay: percentage radioactivity retained on B-6 membrane filter. 0.1 μg φX DNA was used per assay (specific activity 4×10^4 dpm/μg).

when the enzyme was heated at 50 and 65°, respectively, in a 100 m*M* NaCl, 10 m*M* Tris, pH 7.0, buffer.

pH optimum. The pH optimum of the purified DNase was determined in 20 m*M* potassium phosphate solutions. Maximum activity was observed at pH 6.8 with 50 and 15% activities appearing at pH 4.5 and 8.0, respectively.

Effect of cation concentration on enzyme activity. Mg^{2+}, Ca^{2+}, and Zn^{2+} inhibit the purified enzyme activity significantly as shown in Table II.

Specificity for single-stranded DNA. Sea nettle DNase was found to completely digest heat denatured *E. coli* DNA in 100 m*M* NaCl, 10 m*M* Tris, pH 6.8. Very small amounts of acid-soluble material were released from native *E. coli* DNA or DNA of [^{3}H]φX 174 RF1 (Fig. 5). The enzyme also was found to digest heat-denatured φX 174 DNA.

Competition experiments. Varying concentrations of native or heat-denatured calf thymus DNA were added in a competitive experiment to the enzyme mixture while assaying the release of acid-soluble material from heat-denatured [^{3}H]φX 174 RF1. Unlabeled calf thymus DNA did not change the amount of acid-soluble DNA released, but heat-denatured calf thymus DNA did compete with the radiolabeled φX DNA releasing less acid-soluble DNA.

t RNA. The presence of *E. coli* tRNA in the enzyme substrate mixture at concentrations 15-fold greater than the substrates did not produce any appreciable inhibition of purified DNase activity.

Lack in change in activity using ultraviolet-irradiated DNA substrates. Native *E. coli* DNA and φX 174 RF1 DNA were irradiated with different ultraviolet

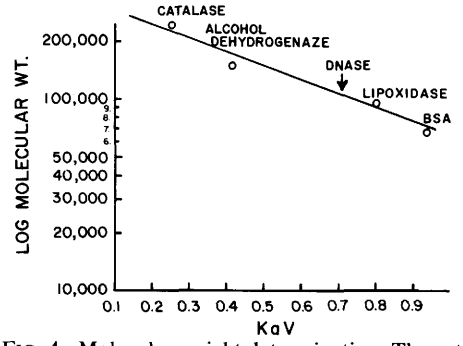

FIG. 4. Molecular weight determination. The active enzyme fraction eluted from hexylamine Sepharose chromatography was applied to a 0.5 M Biogel column and eluted with 50 m*M* potassium phosphate buffer containing 5% glycerol. The enzyme was detected by the release of acid-soluble products from heat-denatured DNA and by the incision assay. Standard proteins of known size were detected by 280 nm absorbance.

TABLE II. INHIBITION OF NETTLE ENDONUCLEASE ACTIVITY BY VARIOUS CATIONS

Cation	Degree of inhibition (%)
1 m*M* $MgCl_2$	10
5 m*M* $MgCl_2$	60
1 m*M* $CaCl_2$	20
5 m*M* $CaCl_2$	95
0.1 m*M* $ZnCl_2$	40
0.25 m*M* $ZnCl_2$	60
0.4 m*M* $ZnCl_2$	99

Note. The reaction mixture was 0.2 ml containing 0.3 nmol *E. coli* heat-denatured DNA in 10 m*M* Tris, pH 7.0, buffer with 20 m*M* NaCl. Incubation time was 30 min at 37°.

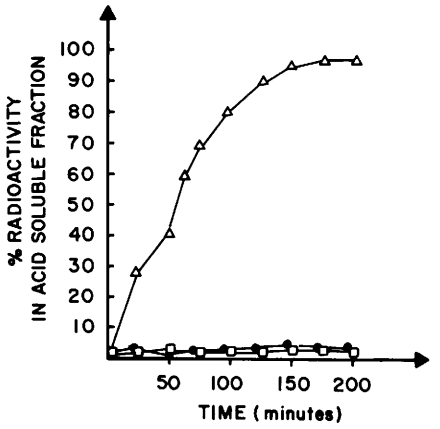

FIG. 5. Time course of digestion of native and denatured *E. coli* DNA and ϕX 174 RF1 DNA. The DNase activity was detected by the release of acid-soluble material from the DNA. The incubation mixture was 10 m*M* Tris, 10 m*M* NaCl, pH 7.0. The amount of DNA used was 0.3 μg per assay and the amount of enzyme per assay column was 0.2 U. ●, ϕX 174 RF1 DNA. △, Heat-denatured *E. coli* DNA. □, Native *E. coli* DNA.

doses up to 2500 erg/mm^2. Incubating these substrates or their nonirradiated counterparts with the enzyme did not result in any variation in the release of acid-soluble material. Similar irradiation of heat-treated ϕX 174 RF1 DNA and *E. coli* DNA did not affect the incision of the nucleic acid by the enzyme.

Stimulation of activity by NaCl. Sea nettle DNase activity was stimulated by increasing NaCl concentrations up to a maximum of 100 m*M* NaCl (Fig. 6). The enzyme remained active with heat-denatured *E. coli* DNA even at 500 m*M* NaCl. On the other hand, when native ϕX 174 RF1 DNA was the substrate and the incision assay was used, a salt concentration of 100 m*M* was sufficient to inhibit the reaction (Fig. 7) by the enzyme.

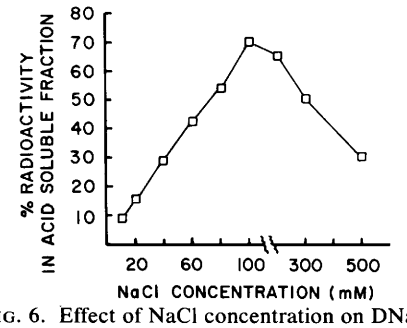

FIG. 6. Effect of NaCl concentration on DNase activity. The DNase activity was determined by the release of acid-soluble material from heat-denatured *E. coli* DNA. The reactions were done in 10 m*M* Tris buffer, pH 7.0. The amount of *E. coli* DNA was 1 μg per assay and the amount of enzyme was 1 unit per assay.

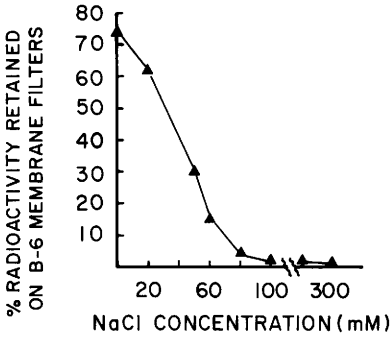

FIG. 7. Effect of NaCl concentration on incision activity of the enzyme on [^{3}H]ϕX 174 RF1 DNA (0.3 μg). 0.5 U of the purified enzyme was used. Controls were run at each NaCl concentration used and showed no inhibition.

Alkaline sucrose sedimentation. Heat-denatured *E. coli* DNA was partially digested under standard conditions. An untreated control and the partially digested samples containing 0.1 and 0.7% acid-soluble material were analyzed on alkaline sucrose gradients. The results shown in Fig. 8 demonstrate a shift in the sedimentation rate of DNA in both enzyme-treated samples.

Chromatographic analysis of end products. Only 0.5% of the DNA was found to be dTMP, while 35% was between $(dT)_2$ and $(dT)_4$. The remaining 65% consisted of higher nucleotides.

Assay of 3'- to 5'-exonucleolytic activity. A tritiated nucleotide was incorporated into the ends of λ phage DNA. The phage DNA was denatured by heat treatment and exposed for varying times up to 50 min to nettle DNase eluted from the hexylamine column. The amount of enzyme in the reaction was sufficient to release 100% of all acid-soluble products from 1 μg heat-denatured *E. coli* DNA. After incubation of 50 min at 37°, there was no significant release of acid-soluble product from the λ phage DNA. A positive control was performed by

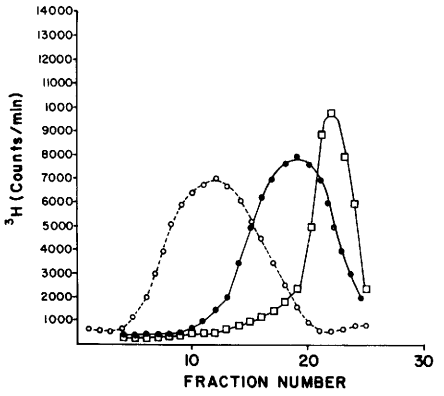

FIG. 8. Alkaline sucrose gradient sedimentation of denatured *E. coli* DNA after partial digestion. Equal amounts of denatured *E. coli* DNA (10 μg with specific activity 10^4 dpm/μg) were added to three reaction mixtures. Purified enzyme (0.2 units) was added to two of the mixtures which were incubated at 37°. The samples contained 0.1% (●) and 0.7% (□) acid-soluble products (2- and 6-min incubation, respectively). The control (○) contained only denatured *E. coli* DNA and was incubated for 6 min at 37°.

the addition of 25 μg of snake venom phosphodiesterase to the nettle enzyme–nucleotide mixture 30 min after the onset of the experiment. Twenty minutes later, 85% of the radioactivity incorporated in the λ phage DNA was released.

Discussion. The enzyme purified in this work is highly specific for single-stranded DNA. Its activity is stimulated by NaCl up to at least 500 m*M* concentration. The enzyme does not appear to require any cofactors and is inhibited by Mg^{2+}, Ca^{2+}, and Zn^{2+}. It is a broad-spectrum DNase, active at wide pH values ranging from 4 to 8, and has an approximate molecular weight of 110,000 daltons.

In low salt concentration, the enzyme incises ϕX 174 RF1 DNA but does not release acid-soluble material from the incised DNA. On the other hand, high sodium chloride concentrations inhibit the incision activity completely. Limited cleavage of superhelical DNA by endonucleases specific for single-stranded polynucleotide chains has been reported by several investigators (23–26). This type of cleavage occurs in the unpaired regions of superhelical DNA (27). Otto *et al.* (26) report that slight modifications of sodium chloride in the mixture can inhibit the incising of ϕX 174 RF1 DNA. This observation was thought to be due to increased enzymatic specificity to single-stranded DNA and thus decreased specificity to double-stranded DNA. It is also possible that fewer single-stranded regions available to the enzyme exist on the superhelical DNA substrate in higher salt concentrations. The incision of ϕX 174 RF1 DNA and the acid-soluble material release of heat-denatured DNA are associated with a narrow fraction range in column separations (Fig. 2), gel electrophoresis, isoelectric focusing (Fig. 3), and molecular weight determination. Thus it appears that a single enzyme is responsible for these activities.

The nettle DNase did not exhibit an increased ability to incise ultraviolet irradiated ϕX 174 RF1 DNA, indicating that it cannot recognize conformational changes in ultraviolet-irradiated DNA. The shift in sedimentation of the sample having 0.1% acid-soluble material is indicative of an endonucleolytic mode of action as the DNA molecules were reduced in size even when a small amount of substrate was acid soluble. The experiments performed to delineate the action of the nettle DNase using radiolabeled end nucleotides or various forms of phosphodiesterase have demonstrated that the DNase lacked 3′- to 5′-exonucleolytic activity and that the cleaved reaction products contain 5′-phosphoryl or 3′-hydroxyl end nucleotides.

The physiological role of the sea nettle endonuclease is not yet known. The properties of this enzyme allowing removal of small DNA pieces from unpaired regions of natural DNA is similar to the action of S_1 endonucleases (25). These properties of the DNase of the sea nettle and other animal venoms could be to increase local damage to the wound site.

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