Isolation and Characterization of an Apurinic Endodeoxyribonuclease from the Anaerobic Thermophile *Desulfotomaculum nigrificans*

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An endodeoxyribonuclease specific to apurinic sites in DNA was purified 12000-fold from vegetative cells of the anaerobic thermophilic bacterium, *Desulfotomaculum nigrificans* strain IFO 13698. The enzyme specifically hydrolyses phosphodiester bonds of apurinic double-stranded DNA, without action on normal, alkylated, or single-stranded depurinated DNA. The endodeoxyribonuclease has a molecular weight of about 18500 and a sedimentation value of 2.2S. The enzyme has an optimum pH of 7.5–8.0 and an optimum temperature of 55 °C. The half-life of purified enzyme is 55 min at 60 °C but it can be heated at 60 °C for at least 60 min without loss of activity in the presence of BSA. The purified enzyme has an absolute requirement for Mg2+ or Mn2+, and is inhibited by EDTA. Enzyme activity is completely inhibited by 0.5 M-NaCl or 1 mM-p-chloromercuribenzoate. All these properties differ from those of apurinic/apyrimidinic endodeoxyribonucleases isolated from *Bacillus subtilis*, *Bacillus stearothermophilus*, and *Escherichia coli*.

INTRODUCTION

It is generally recognized that DNA spontaneously loses purines (Lindahl & Nyberg, 1972) and pyrimidines (Lindahl & Karlström, 1973). The rate of base loss is considerably increased by treatment with chemicals such as acid, alkylating agents and carcinogens, or by exposure to UV and X-rays (Lindahl, 1979).

A mechanism is proposed for repair of depurinated DNA. An endonuclease specific to apurinic/apyrimidinic sites (APendodeoxyribonuclease) recognizes the site and incises the phosphodiester chain near the lesion, which is subsequently removed by an exonuclease (Lindahl, 1979). The gap thus formed is filled by a DNA polymerase, and the phosphodiester chain continuity is restored by a polynucleotide ligase. APendodeoxyribonuclease was first discovered in *Escherichia coli* (Verly & Paquette, 1972). This enzyme has been found in other bacteria (Bibor & Verly, 1978; Clements et al., 1978; Hecht & Thielman, 1978; Inoue & Kada, 1978; Ljungquist, 1977; Pierre & Laval, 1980; Sako et al., 1980a; Verly & Rassart, 1975), in animals (Brent, 1976; Kane & Linn, 1981; Linsley et al., 1977; Ljungquist & Lindahl, 1974; Teebor & Dukor, 1975; Thibodeau et al., 1980) and in a plant (Svachilova et al., 1978). Recently we reported the purification and characterization of a new magnesium-dependent APendodeoxyribonuclease from vegetative cells of *Bacillus subtilis* (Sako et al., 1980a, b; Sako et al., 1981).

Apurinic/apyrimidinic sites on DNA molecules are also introduced at high temperature (Greer & Zamenhof, 1962). It is calculated that the rate of DNA depurination (k) at pH 7.4 and 70 °C is about 4 x 10⁻⁹ s⁻¹ (Lindahl & Nyberg, 1972), i.e. about 50 purine residues are lost from the bacterial DNA per generation at 70 °C. This is a serious problem for thermophilic bacteria.

*Abbreviation*: APendodeoxyribonuclease, apurinic/apyrimidinic endodeoxyribonuclease.
There is only one report (Bibor & Verly, 1978) which describes the APendodeoxyribonuclease of a thermophilic bacterium (*Bacillus stearothermophilus*). We have looked for an endonuclease hydrolysing a phosphodiester bond near apurinic/apyrimidinic sites in the anaerobic thermophilic spore-forming bacterium *Desulfotomaculum nigrificans*, which grows at 55 °C. The enzyme was detected, purified and characterized.

**METHODS**

*Bacterial strain and growth conditions. Desulfotomaculum nigrificans* IFO 13698 was obtained from the Institute for Fermentation, Osaka, Japan and grown at 55 °C in PPP medium based on that of Brewer (1940) containing: 17 g Polypepton, 3 g phytone peptone, 6 g sodium pyruvate, 2.5 g NaCl, 0.5 g sodium thioglycollate, 0.25 g L-cystine, and 1 g Na₂SO₄, adjusted to pH 7.0 in 1 litre of distilled water. Stock cultures were maintained in liquid media at 55 °C and subcultured every two weeks. *Desulfotomaculum nigrificans* was grown in 101 carboys which were filled to the neck with PPP medium to obtain anaerobiosis. Each carboy was inoculated with 500 ml of a 40 h starter culture and incubated at 55 °C for about 39 h. Cells were collected by continuous centrifugation in a Tomy RS20-III centrifuge.

**Preparation of substrates. Bacillus subtilis* 168Tt (thy *trp*) was grown in Schaeffer liquid nutrient broth (Kadota et al., 1978) containing 0.5 µCi of [Me-³H]thymidine ml⁻¹ (53 Ci mmol⁻¹; 1.961 TBq mmol⁻¹) at 37 °C, and was harvested at late exponential phase. DNA was extracted according to the method of Marmur (1961). Purified DNA was dissolved in SSC solution (0.15 m-NaCl, 0.015 m-sodium citrate, pH 7.0) and stored at −20 °C.

In order to prepare sonicated [³H]DNA, native [³H]DNA was sonicated for 5 min (1 min bursts at 30 s intervals) at 0 °C with a Tomy Ultrasonic disruptor UR 200P at the lowest output (20 W).

Alkaline DNA was dissolved in SSC solution (0.15 m-NaCl, 0.015 m-sodium citrate, pH 7.0) and stored at −20 °C. In order to prepare single-stranded [³H]DNA or single stranded depurinated [³H]DNA, native [³H]DNA or alkylated–depurinated [³H]DNA was dissolved against 90% (v/v) formamide, 5 m-mammotassium phosphate (pH 6.8) and was heated for 2 h at 37 °C. After chilling, DNA solutions were again dialysed against 1.5% formaldehyde, 5 m-sodium phosphate (pH 6.8), and were applied to a hydroxyapatite column to isolate single-stranded DNA as described by Miyazawa & Thomas (1965). After a treatment of this single-stranded depurinated [³H]DNA with alkaline solution, about 40% of the alkylated–depurinated [³H]DNA became acid-soluble. The specific activity of these labelled DNA substrates was about 19500 d.p.m. µg⁻¹.

**Endonuclease assay.** Endonuclease activity was determined by the modification of a method previously described (Sako et al., 1980). Alkylated–depurinated DNA (1-µg) labelled with [Me-³H]thymidine (19600 d.p.m. µg⁻¹) in 20 µl SSC (pH 7.0), 10 µl 100 mm-MgCl₂ buffer A (50 mm-Tris/HCl, 0.1 mm-EDTA, 0.1 mm-2-mercaptoethanol, 10% (v/v) glycerol, pH 8.0), 50 µl buffer A were mixed with 20 µl of enzyme solution in buffer A. After incubation at 55 °C for 15 min, the tubes were chilled and 200 µl SSC containing 400 µg calf thymus DNA and 200 µl 12.5% (v/v) perchloric acid were successively added to each tube to stop the enzyme reaction. The mixtures were allowed to stand at 0 °C for 15 min and centrifuged at 13000 for 10 min. The radioactivity in the supernatant was determined in toluene/Triton X-100 scintillation fluid in a liquid scintillation counter at a counting efficiency of 33%. One unit of enzyme activity was defined as the amount of enzyme activity necessary to release 10% (acid-soluble) radioactivity of the substrate.

**Sedimentation coefficient determination.** The sedimentation coefficient and molecular weight of the APendodeoxyribonuclease were determined by glycerol gradient centrifugation by the modified method of Martin & Ames (1961). Samples of purified enzyme dialysed against buffer B (50 mm-Tris/HCl, 0.1 mm-EDTA, 0.1 mm-2-mercaptoethanol, 7% glycerol, pH 8.0) were sedimented through a linear 10 to 30% glycerol gradient in buffer A. Centrifugation was performed at 4 °C for 24 h at 38000 r.p.m. in a Beckman SW 50.1 rotor. The gradients were fractionated with an ISCO density gradient fractionator (model 185) and a UV-absorbance monitor (model UA-5). APendodeoxyribonuclease activity in each fraction was assayed. Cytochrome c, trypsin inhibitor, chymotrypsinogen A, ovalbumin, and BSA were used as marker proteins.

**PAGE.** Electrophoresis of native enzyme was carried out with 7.5% (w/v) polyacrylamide gels as described by Davis (1964). The sample was applied to the stacking gel and the electrophoresis was carried out in 0.025 m-Tris/0.2 m-glycine buffer, pH 8.3, at 3 mA. After electrophoresis at 4 °C, one of the gels was stained with Coomassie brilliant blue for detection of protein and a duplicate gel was cut in 3 mm slices. The slices were eluted for 16 h at 4 °C with 0.2 ml buffer A and APendodeoxyribonuclease activity was measured.

**Protein determination.** For column chromatography, the protein was routinely determined by measuring absorbance at 280 nm. In experiments where specific activity was determined, protein was measured by the Lowry method using BSA as standard.
Chemicals. [Me-3H]Thymidine (53 Ci mmol⁻¹; 1.961 TBq mmol⁻¹) was purchased from New England Nuclear. DEAE-cellulose (DE52) was obtained from Whatman. Sephadex G-200 was obtained from Pharmacia. Cellulose and calf thymus DNA (type 1) were Sigma products. Polypepton was obtained from Daigo, Osaka, Japan, and beef extract from Kyokuto, Tokyo, Japan. All other chemicals were obtained from Nakarai Chemical Co., Kyoto, Japan.

RESULTS AND DISCUSSION

Purification of APendodeoxyribonuclease

(i) Preparation of crude cell extracts. Desulfotomaculum nigrificans IFO 13698 was grown as described in Methods. The cells were harvested at late exponential growth phase by centrifugation, washed twice with buffer C (0.01 M-Tris/HCl, 0.06 M-NH₄Cl, 0.01 M-magnesium acetate, and 0.07 M-2-mercaptoethanol, pH 7.5), and the pellet was stored at −20 °C. Thawed cell paste (91 g wet wt) was suspended in 180 ml buffer A. Cells were disrupted by two passages through a French press (2000 kg cm⁻²; Ohtake, Japan). The suspension was centrifuged at 16000 g for 40 min at 4 °C and the supernatant was collected (preparation I).

(ii) Ultracentrifugation fractionation. Preparation I was centrifuged at 80000 g for 120 min in a Spinco type 30 rotor at 4 °C. Then the supernatant fraction was collected and diluted by adding 100 ml buffer A (preparation II).

(iii) Ammonium sulphate precipitation. To preparation II, solid ammonium sulphate was added slowly to reach 40% saturation. After centrifugation at 16000 g for 30 min, the supernatant was brought to 80% saturation. After centrifugation, the pellet was dissolved in 130 ml buffer A and dialysed against the same buffer for 10 h at 4 °C (preparation III).

(iv) DEAE-cellulose chromatography. Preparation III was applied to a column (4.0 × 30 cm) of DEAE-cellulose equilibrated with buffer A which was then washed with 350 ml buffer A. A 4 l volume of a 0 to 0.5 M-NaCl linear gradient in buffer A was applied at a flow rate of 120 ml h⁻¹ and 20 ml fractions were collected. Several peaks of APendodeoxyribonuclease activity were observed (Fig. 1). The bulk of the enzyme activity was eluted between 0.06 and 0.1 M-NaCl in fractions 66 to 78. These were pooled (preparation IV) and concentrated to 30 ml with a Millipore ultrafiltration apparatus with a PSAC filter (mol. wt 1000 exclusion) and concentrated to 4 ml with Collodion bags (Sartorius).

(v) Sephadex G-200 gel filtration. Concentrated preparation IV (4 ml) was applied to a column (2.6 × 95 cm) of Sephadex G-200 equilibrated with buffer A. The sample was eluted with the same buffer at a rate of 12 ml h⁻¹, and the eluent was collected in 4 ml fractions. The
enzyme was separated from the bulk of the protein (Fig. 2). Fractions 88 to 100 were pooled (preparation V).

(vi) DNA-cellulose chromatography. Preparation V (50 ml) was concentrated to 2 ml with Collodion bags and applied to a DNA-cellulose column (0.8 x 5 cm) prepared according to the method of Litman (1968) and equilibrated with buffer A. The column was washed with 40 ml of the same buffer, and eluted with buffer A containing 0.4 M-NaCl, at a flow rate of 3 ml h⁻¹; 2 ml fractions were collected (Fig. 3). Fractions 21 to 23, showing activity, were pooled and dialysed against buffer A (preparation VI). The glycerol concentration was raised to 40% and the solution was kept at -20 °C. At this stage, however, the enzyme was very labile and the activity was frequently lost within a week.

The procedure for purification of APendodeoxyribonuclease from D. nigrificans is summarized in Table 1. The APendodeoxyribonuclease was purified about 12000-fold, and the overall yield of the activity was 3.9%.

The purity of the enzyme was determined by PAGE. Electrophoresis revealed a single protein band which corresponded to the enzyme activity (Fig. 4).

All purification procedures were performed at 0 to 4 °C unless otherwise indicated.
Fig. 4. PAGE of preparation VI of APendodeoxyribonuclease. Duplicate gels were used. Details of the experimental procedure are given in Methods. A photograph of the gel stained with Coomassie brilliant blue is shown in the upper part of the figure. APendodeoxyribonuclease activity in slices of a parallel gel is given at the bottom. Migration is from left to right.

Table 1. Summary of purification of D. nigriJicans endodeoxyribonuclease for apurinic sites

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Volume (ml)</th>
<th>Total protein (mg)</th>
<th>Endonuclease (units)</th>
<th>Yield (%)</th>
<th>Specific activity (units mg⁻¹)</th>
<th>Purification factor (fold)</th>
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<td>I Crude extract</td>
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<td>6273</td>
<td>15249</td>
<td>100</td>
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<td>1</td>
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<tr>
<td>II Ultracentrifugation</td>
<td>700</td>
<td>5012</td>
<td>7910</td>
<td>51</td>
<td>1.6</td>
<td>0.6</td>
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<tr>
<td>III Ammonium sulphate</td>
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<td>4346</td>
<td>7261</td>
<td>47</td>
<td>1.7</td>
<td>0.7</td>
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<td>IV DEAE-cellulose</td>
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<td>112</td>
<td>5837</td>
<td>38</td>
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<td>21</td>
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<tr>
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<td>9.5</td>
<td>583</td>
<td>242</td>
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<tr>
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<td>0.02</td>
<td>606</td>
<td>3.9</td>
<td>30300</td>
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Properties of APendodeoxyribonuclease

Temperature optimum. The effect of temperature on the enzyme activity of D. nigriJicans (preparation VI) was compared with that of B. subtilis (preparation VI) (Sako et al., 1980a) (Fig. 5). The optimum temperature for the enzyme activity of thermophile D. nigriJicans was 55 °C, higher than that of the purified enzyme (preparation VI) of B. stearothermophilus which was 45 °C (Bibor & Verly, 1978).

pH optimum. The purified enzyme was active between pH 6.5 and 9.0, with a slight maximum at pH 7.5 to 8.0. The enzyme of D. nigriJicans had a broader pH optimum than other APendodeoxyribonucleases.

Molecular weight. A single symmetrical peak of enzyme activity sedimented in the glycerol gradient. In comparison with the five marker proteins, the sedimentation coefficient of APendodeoxyribonuclease was calculated to be 2.2S (Fig. 6). Assuming the enzyme to be spherical, the molecular weight of APendodeoxyribonuclease is estimated to be 18 500 according to the method devised by Martin & Ames (1961). The molecular weight of purified enzyme from D. nigriJicans is smaller than those of B. subtilis enzymes. Two kinds of APendodeoxyribonuclease of B. subtilis have been reported. One of them has a molecular weight of 56 000 (Inoue & Kada, 1978) and the other a molecular weight of 105 000 and comprising four identical subunits (Sako et al., 1980a). Moreover, the molecular weights of the APendodeoxyribonuclease from E. coli (Ljungquist, 1977; Verly & Rassart, 1975), B. stearothermophilus (Bibor & Verly, 1978), Micrococcus luteus (Hecht & Thielman, 1978; Pierre & Laval, 1980), Haemophilus influenzae
Fig. 5. Action of incubation temperature on purified enzymes of *D. nigrificans* and *B. subtilis*. Preparation VI samples of *D. nigrificans* and *B. subtilis* (Sako et al., 1980a) APendonuclease (1.5 units) were used. The endonuclease activity was assayed under the standard assay conditions, except that the incubation temperature was varied. The maximum activity of each enzyme was scored as 100%. O, *D. nigrificans* enzyme; •, *B. subtilis* enzyme.

Fig. 6. Determination of molecular weight of APendonuclease by glycerol gradient centrifugation. The distances in the glycerol gradients are plotted as a function of their sedimentation coefficients. The sedimentation coefficient ($s_{20,w}$) and molecular weight of the standard proteins are: BSA, 4.6S (67000); ovalbumin, 3.66S (43000); chymotrypsinogen A, 2.54S (25000); trypsin inhibitor, 2.3S (20100); cytochrome c, 1.8S (12400).

(Clements et al., 1978), *Saccharomyces cerevisiae* (Thielman & Hess, 1981), calf thymus (Ljungquist & Lindahl, 1974), calf liver (Kuebler & Goldthwait, 1977), placenta (Linsley et al., 1977), and HeLa cells (Kane & Linn, 1981) are approximately 30000. However, the molecular weights of APendonuclease from barley leaves (Svachilova et al., 1978) and rat-liver chromatin (Thibodeau et al., 1980) are 18000 and 12500, respectively.

Substrate specificity. In order to study the substrate specificity, preparation VI was mixed with 3H-labelled untreated DNA, single-stranded untreated DNA, sonicated DNA, alkylated DNA, or double-stranded alkylated–depurinated DNA and incubated at 55°C. Preparation VI has an activity specific to double-stranded alkylated–depurinated DNA, and has little activity on native, single-stranded, sonicated, and alkylated DNAs (Fig. 7a). Therefore, *D. nigrificans* APendonuclease does not show detectable exonuclease activity, in common with APendonuclease from barley leaves (Svachilova et al., 1978) and rat-liver chromatin (Thibodeau et al., 1980) are 18000 and 12500, respectively.

In order to study the APendonuclease activities on the apurinic sites in single-stranded DNA, single-stranded alkylated–depurinated DNA was prepared as described in Methods. However, preparation VI had little activity on this substrate (Fig. 7b). Thus, the specificity of this enzyme was very similar to others from many sources and we conclude that APendonuclease from *D. nigrificans* has an activity specific to double-stranded depurinated DNA.

Effects of divalent cations and EDTA. In order to study the effect of EDTA and divalent cations, preparation VI was dialysed against buffer D (0.05 M-Tris/HCl, pH 8.0, 0.1 M-2-mercaptoethanol, 10% glycerol) to remove EDTA. APendonuclease activity on alkylated–depurinated [*H]DNA was measured as a function of the concentration of Mg$^{2+}$, Mn$^{2+}$, and Ca$^{2+}$ (Table 2). The purified enzyme strictly required Mg$^{2+}$ or Mn$^{2+}$ for its activity. The optimum concentration was 10 mM for MgCl$_2$ and between 10 and 20 mM for MnCl$_2$; higher concentrations of both cations inhibited the enzyme. Ca$^{2+}$ was not effective. When the reaction mixture contained 10 mM-MgCl$_2$, 5 mM-EDTA did not inhibit the enzyme activity.
D. nigrijicans apurinic endonuclease

Fig. 7. Activities of the purified enzyme (preparation VI) on native, denatured, sonicated, alkylated, double-stranded depurinated and single-stranded depurinated DNA. (a) A sample (200 μl, 10 units) of preparation VI in buffer A was mixed with 200 μl SSC, 20 mM-MgCl₂, pH 7-0 buffer containing 11 μg [³H]DNA which was either native (△), denatured (□), sonicated (●), alkylated (○), or double-stranded depurinated (●); the mixtures were incubated at 55 °C. Aliquots were taken from 0 to 60 min to measure the acid-soluble radioactivity which was plotted as fraction of the total radioactivity in the aliquot. (b) Single-stranded depurinated DNA was prepared as described in Methods. Various amounts of enzyme (preparation VI) were mixed with 1.1 pg [³H]DNA which was either double-stranded depurinated (○, △), or single-stranded depurinated (●, △); 10 mM-MgCl₂ was either present (filled symbols) or absent (open symbols). The assays were done in the standard reaction mixtures. However, 20 mM-EDTA inhibited the enzyme activity completely (Table 2). When 20 mM-CaCl₂ was added to the reaction mixture containing 10 mM-MgCl₂, about 50% of maximum activity was retained.

Bacillus subtilis APendodeoxyribonuclease (Sako et al., 1980a) also requires Mg²⁺ for its activity; Mn²⁺ or Ca²⁺ cannot substitute. The divalent cation requirements of the enzyme are different from those of the enzymes isolated from the other prokaryotes (Clements et al., 1978; Inoue & Kada, 1978; Ljungquist, 1977; Pierre & Laval, 1980; Verly & Rassart, 1975) or eukaryotes (Linsley et al., 1977; Thielman & Hess, 1981) where no absolute requirement for divalent cations was found. On the other hand, APendodeoxyribonuclease of B. stearothermophilus requires monovalent cations for optimal activity, is insensitive to EDTA, and is inhibited by divalent cations (Bibor & Verly, 1978). The divalent cation requirements of APendodeoxyribonuclease from D. nigrijicans are similar to those of the enzyme from HeLa cells (Kane & Linn, 1981) and rat-liver chromatin (Thibodeau et al., 1980).

Effect of NaCl. The effects of ionic strength on APendodeoxyribonuclease activities of D. nigrijicans (preparation VI) and B. subtilis (preparation VI) (Sako et al., 1980a) were compared. The enzyme activity of B. subtilis was unusually resistant to the presence of NaCl in the reaction mixture. About 60% of maximum activity was retained in the presence of 0·5 M-NaCl though the activity was completely inhibited by 1 M-NaCl. However, the enzyme activity of D. nigrijicans was more sensitive to NaCl than that of B. subtilis. About 50% of maximum activity was retained in the presence of 0·05 M-NaCl, and the activity was completely inhibited by 0·5 M-NaCl (Fig. 8). Since E. coli endonuclease IV was 50% inhibited by 0·56 M-NaCl (Ljungquist, 1977), the enzyme of D. nigrijicans is more sensitive to NaCl than that of E. coli.

Effect of other small molecule compounds. Dithiothreitol and 2-mercaptoethanol (1 to 10 mM) had at most only a slight stimulatory effect (10 to 20%) on enzyme activity. Activity was completely inhibited by addition of 1 mM-p-chloromercuribenzoate but was not influenced by
Table 2. Effects of divalent cations and EDTA on APendodeoxyribonuclease activity

For the first experiment, 20 µl depurinated DNA (1-1 µg) in SSC (pH 7-0) and 20 µl preparation VI (2 units) dialysed against buffer D (0-05 M-Tris/HCl, pH 8-0, 0-1 mm-2-mercaptoethanol, 10% glycerol) were mixed with 60 µl buffer D; MgCl₂, MnCl₂, or CaCl₂ was added to reach the indicated concentration. For the second experiment, 20 µl depurinated DNA (1-1 µg), 20 µl preparation VI (two units) dialysed against buffer D, and 10 µl 100 mm-MgCl₂ in buffer D were mixed with 50 µl buffer D; EDTA was added to reach the indicated concentration. After incubation at 55 °C for 15 min, radioactivity in the acid-soluble fraction was measured.

<table>
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<th>Experiment</th>
<th>Addition</th>
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* EDTA was absent in all reaction mixtures.
† All the mixtures contained 10 mm-MgCl₂.

Fig. 8. Relative activity of APendodeoxyribonuclease from D. nigrificans and B. subtilis at different NaCl concentrations. Preparation VI samples of D. nigrificans and B. subtilis (Sako et al., 1980a) APendodeoxyribonuclease (1-5 units) were used as enzyme solution. The activity without NaCl was scored as 100%. ○, D. nigrificans enzyme; ●, B. subtilis enzyme.
addition of 5 mM-N-ethylmaleimide. The addition of 0.005 to 0.05% Triton X-100 to the reaction mixture caused a little stimulation (10 to 20%) of enzyme activity.

Thermosensitivity. Figure 9 shows the thermosensitivity of the enzyme activity at 60 °C. Thermostability of purified enzyme of *D. nigrificans* was higher than that of *B. stearothermophilus*. The rate of denaturation of preparation VI in buffer A followed first order kinetics with a \( t_\text{1/2} \) of 34 min at 60 °C. However, the \( t_\text{1/2} \) of preparation IV was about 55 min at 60 °C. The addition of 0.2% BSA to preparation VI protected the enzyme so that it could be incubated at 60 °C for at least 60 min without loss of activity. The \( t_\text{1/2} \) of *B. stearothermophilus* enzyme was 11 min at 60 °C (Bibor & Verly, 1978).

Previously, it was believed that an apurinic site in DNA was more likely to be an inactivating rather than mutagenic lesion (Drake & Baltz, 1976). Recently it has been recognized that under certain conditions, for instance SOS-induction, depurination can be mutagenic (Kunkel *et al.*, 1981; Schaaper & Loeb, 1981; Shearman & Loeb, 1979). It is thought, therefore, that APendodeoxyribonuclease may play an essential role in the growth of *D. nigrificans* at higher temperature.

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