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Preparation and Properties of a New DNase from *Aspergillus oryzae*†

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ABSTRACT: A DNase present in commercial preparations of *Aspergillus oryzae* α-amylase was purified 1550-fold in 25% yield by acetone precipitation and by chromatography on diethylaminoethyl- and carboxymethylcellulose. The enzyme was isolated free of contaminating RNases and DNases. The molecular weight of the enzyme determined by gel filtration on Sephadex G-100 was 48 000, while a molecular weight of 58 000 was determined for the single band observed upon polyacrylamide gel electrophoresis in sodium dodecyl sulfate. The isoelectric point of the DNase is 9.2. The enzyme hydrolyzed only DNA with a pH optimum of 8.2 and was activated by Co^{2+}, and to a lesser extent by Mg^{2+} and Mn^{2+}. Native DNA was a better substrate than heat-denatured DNA. Enzymatic digests of calf thymus and *E. coli* DNA yielded oligomers of chain lengths ranging from 10 to 200, with mono- and small oligonucleotides (chain length less than 5) detected only when large (100 mg) amounts of DNA were fractionated by column chromatography on diethylaminoethyl-Sephadex A-25 in 7 M urea. The digestion products contained 5′-terminal phosphate groups and mostly adenosine at the 3′ and guanosine and adenosine at the 5′ ends.

DNases are useful tools for the study of the structure of nucleic acids. Their application depends on the specificity of the particular nuclease. Thus, restriction enzymes cleave DNAs at a defined sequence of residues (Nathans and Smith, 1975). Conformation-specific enzymes such as S1 nuclease hydrolyze only single-stranded substrates (Ando, 1966; Vogt, 1973). Nonspecific nucleases may be used for a variety of purposes. For example, micrococcal nucleases, rodent liver endogenous endonuclease, DNase I, and DNase II have been used in many studies of chromatin structure (Clark and Felsenfeld, 1971; Gottesfeld et al., 1975; Hewish and Burgoyne, 1973; Noll, 1974a; Simpson and Whitlock, 1976a; Sollner-Webb et al., 1976). With such nonspecific nucleases, the influence of the physicochemical properties of the enzymes on their interaction with nucleoprotein substrates may also be studied.

As described here, we have purified a new DNase from commercial preparations of crude amylase from *Aspergillus oryzae*. The enzyme has catalytic properties similar to DNase I, but differs from it with respect to its molecular weight and isoelectric point of pH 9.2. The latter property facilitates the purification of the DNase by ion-exchange chromatography, giving a 1550-fold purification with a 25% yield.

This DNase differs from other enzymes (nucleases 0, S1, K1, and K2) derived from the same mold by one or more of the following parameters: molecular weight, isoelectric point, preferential hydrolysis of native rather than denatured DNA, or lack of activity toward RNA (Kato and Ikeda, 1968; Suzuki and Sakaguchi, 1974; Ando, 1966; Uozumi et al., 1968). The DNase can be used to generate oligonucleotides about 10 bases

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in length, which may be potentially useful in understanding the association of DNA with histones, leading to the folding and compaction of the DNA within the cell nucleus.

Experimental Section

Adsorbents. DEAE[1]-cellulose (0.8 mequiv/g) and CM-cellulose (0.64 mequiv/g) were obtained from Schleicher and Schuell. DEAE-Sephadex A-25 and Sephadex G-100 and G-200 were obtained from Pharmacia. Columns were equilibrated until the effluent and influent pH and conductivity were the same.

Enzymes. Alkaline phosphatase from *E. coli* (Sigma, type III) was further purified on DEAE-cellulose (Weiss et al., 1968) and used as described by Neu and Heppel (1965). Pancreatic RNase (type II A) and RNase T_2 (grade VI) were obtained from Sigma and assayed at pH 7.5 or 4.5, respectively, as previously described (Rushizky and Sober, 1963). Spleen phosphodiesterase (Type SPH), micrococcal nuclease (NFCP), and DNase I (DSV) were from Worthington, and snake venom phosphodiesterase from Pierce Chemical Co. S1 nuclease was prepared as previously reported (Rushizky et al., 1975). Crude α-amylase, hemoglobin, and bovine serum albumin were obtained from Sigma.

Nucleic Acids. T7 phage, grown on *E. coli* B, was purified (Yamamoto et al., 1970) and the DNA isolated according to Studier (1965). *E. coli* B DNA was labeled with ^{32}P (Potter and Newbold, 1976; Kelly et al., 1965) and isolated according to Marmur (1961). Calf thymus DNA was from Worthington. Pure $tRNA^{Arg}$ (lot No. 15-141) was provided by Dr. A. D. Kelmers, and Hela 5S RNA by Dr. R. L. Seale. RNA of the *E. coli* phage MS2 was prepared as previously described (Strauss and Sinsheimer, 1963).

Measurement of DNase Activity. Spectrophotometric measurements were made in cells with a 1-cm light path and are expressed as absorbancy (A). To 3.3 mL of a solution containing 40 μg of T7 DNA per mL in 40 mM Tris-HCl (pH 8.2)-1 mM $MgCl_2$ was added 0.2 mL of enzyme diluted in H_2O, and the mixture was incubated at 37 °C for 30 min. After cooling in ice water for 5 min, 0.4 mL of 60% $HClO_4$ was added and the precipitate removed by centrifugation for 10 min at 0 °C. As a blank, the same enzyme dilution was added to 3.3 mL of assay solution plus 0.4 mL of 60% $HClO_4$. A difference of 1.0 between the A_{260} of the supernatants is designated as 1 enzyme unit. At least three enzyme dilutions were tested per assay. The assay was linear for at least 30 min at 37 °C and to A_{260} values of 0.65. For comparison, DNase I was assayed under the same conditions.

During the early stages of enzyme purification (the acetone precipitation, see below), the above assay was complicated by high blank values and/or presence of enzyme inhibitors. Therefore, to better quantitate enzyme recovery, samples to be tested for DNase activity were treated batchwise with DEAE- and then with CM-cellulose (see enzyme purification below). Thus, 6 mL of enzyme in 20 mM Tris-HCl (pH 7.3)-1 mM mercaptoethanol (buffer A) was mixed with 0.5 mL (packed volume) of DEAE-cellulose equilibrated with buffer A. After centrifugation, the supernatant was removed and the adsorbent washed with 6 mL of buffer A. The pooled eluates were mixed with 0.5 mL of CM-cellulose in buffer A and the enzyme activity desorbed with two 3-mL volumes of buffer A containing 0.5 M NaCl. After dilution to 0.1 M NaCl, enzyme activity was assayed as above.

The presence of DNase activity was also determined both by electrophoresis of digestion products in 1.4% agarose slab gels, stained with ethidium bromide (Sharp et al., 1973), and by viscometry (Smith and Wilcox, 1970) with the Beckman low shear viscometer (Zimm and Crothers, 1962). Because of the low DNA concentration (40 μg/mL) in the assay, the same assay solution was used for acid solubility measurements, gel electrophoresis, and viscometry.

Purification of DNase. Ninety grams of crude α-amylase was stirred at 23 °C for 2 h in 500 mL of buffer A. A yellow precipitate was removed by centrifugation at 2000g for 10 min; the supernatant was diluted to 1100 mL with buffer A and adjusted to pH 7.3 and solid Tris. The conductivity of the solution was then equal to that of buffer A. All subsequent steps were carried out at 5-8 °C. The brown solution was applied at a flow rate of 180 mL/h to a 5 × 86 cm DEAE-cellulose column equilibrated with buffer A, and eluted with the same buffer until the effluent A_{280} was below 0.1. Two volumes of acetone was added to the pooled eluate and the precipitate was recovered immediately by centrifugation for 5 min at 2000g. The precipitate was dissolved in 400 mL of buffer A and dialyzed against two 10-L volumes of buffer A for 16 h each. A small amount of precipitate was removed by centrifugation as above, and the supernatant (610 mL) was applied to a 4 × 50 cm CM-cellulose column at a flow rate of 100 mL per h. The column was washed with 1.5 column volumes of buffer A and developed with a 4-L linear gradient of buffer A containing 0-0.5 M NaCl. A small amount of material absorbing at 280 nm (used as a measure of protein content) eluted before the gradient started. There was no other A_{280} material except for that coincident with the enzyme activity peak (max A_{280}/mL = 0.006) at 0.45 gradient volume. To concentrate the enzyme, pooled fractions were dialyzed exhaustively against water and lyophilized. Enzyme activity was stable to storage for at least 3 months at 4 °C at an A_{280}/mL of more than 0.1 after addition of 15% glycerol and 1 mM mercaptoethanol.

Determination of the Physical Properties of DNase. The isoelectric point of the enzyme (Vesterberg, 1970) was determined with 1% ampholytes of pH 3-10 and equipment from LKB as described (Rushizky et al., 1975) using 32 mL of enzyme (14.9 units, total A_{280} of 9.46) in the dense solution. Constant current levels were obtained after 48 h of 500 V.

Estimation of the molecular weight by gel filtration on G-100 was based on the linear curve obtained from the elution volume vs. the logarithm of the molecular weight (Andrews, 1964) of pancreatic RNase, RNase T_2 (Uchida, 1966), and hemoglobin (molecular weights of 13 700, 36 200, and 64 500, respectively). The molecular weight of the DNase was also obtained by discontinuous sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis, using a 3% acrylamide-0.08% bisacrylamide stacking gel, an 18% acrylamide-0.16% bisacrylamide separating gel, and the buffer system of Le Stourgeon and Rusch (1973). The gel was run at a constant current of 20 mA, stained for 1 h in 0.2% Coomassie blue in 50% methanol-7% acetic acid, and destained in 20% methanol-7% acetic acid. Bovine albumin (mol wt 67 000), ovalbumin (mol wt 45 000), myoglobin (mol wt 17 800), and cytochrome c (mol wt 12 400) were used as standards. The DNase was concentrated about 200-fold by dialysis against dry Sephadex G-200 before electrophoresis.

Determination of the Catalytic Properties of DNase. The pH optimum was determined with Mes ((*N*-morpholino)-ethanesulfonic acid) buffers between pH 5.5 and 6.8, Tris-Cl

[1] Abbreviations used: DEAE, diethylaminoethyl; CM, carboxymethyl; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; Mes, 2-(*N*-morpholino)ethanesulfonic acid.

TABLE I: Purification of *Aspergillus* DNase from 90 Grams of Crude Amylase (Sigma).[a]

	Total protein as A_{280}	Total enzyme units	Sp act.	Recovery
Crude extract	173 000	3170	0.018	100%
After DEAE-cellulose	56 000	3510	0.062	110
After acetone pptn and dialysis	14 300	1500	0.105	47
After CM-cellulose	28	788	28	25

[a] For a description of enzyme units and enzyme assay, see text. Specific activity is defined as total units of enzyme/total A_{280}, where A_{280} is used as a measure of protein content.

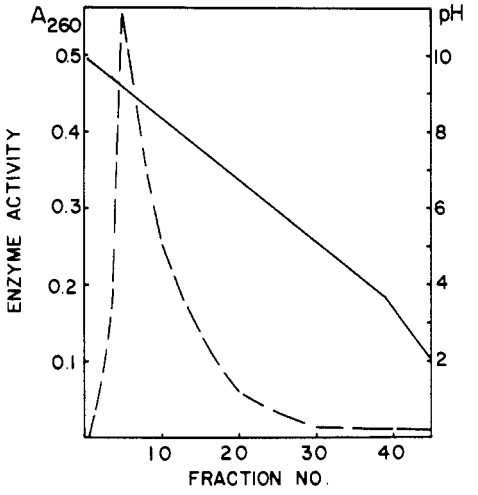

FIGURE 1: Isoelectric focusing of the *Aspergillus* DNase with 1% ampholytes of pH 3–10. The fractions were assayed for pH (-) and for enzyme activity (- - -) by release of acid-soluble A_{260} material.

buffers at pH 7.5–9.3, and sodium borate at pH 8.5. Substrate inhibition of the DNase was measured with 40 μg/mL, 250 μg/mL or 1000 μg/mL of T7 DNA in the assay solution. Metal requirements, the effect of EDTA, and 0.3 M NaCl or 0.01 M sodium phosphate were tested with DNase solutions exhaustively dialyzed against H_2O. SH requirements were also examined with dialyzed DNase solutions, held with 10 mM mercaptoethanol for 72 h at 4 °C (Hanson and Farley, 1969). Activity toward RNA was tested with 40 μg/mL MS2 RNA in place of T7 DNA, and toward denatured DNA with T7 DNA that had been boiled for 30 min in 40 mM Tris-HCl, pH 8.2, and cooled in ice. The purified DNase was checked for phosphatase and nonspecific phosphodiesterase activity by the method of Koerner and Sinsheimer (1957).

Characterization of the Products of DNase Digestion. All digestions were carried out for 3 h at 37 °C in 40 mM Tris-HCl, pH 8.2, 1 mM $MgCl_2$, or $CoCl_2$. An aliquot (0.6–1.0 × 10^6 cpm) of ^{32}P-labeled *E. coli* DNA (1.5 × 10^5 cpm/μg) was incubated with 0.02 and 0.004 unit of DNase per mg of DNA in a 50-μL volume and the digest fractionated on a 12% acrylamide (acrylamide–bisacrylamide 19:1, Cyanogum 41) slab gel in 7 M urea at 25 °C (Maniatis et al., 1975). Bromophenol blue, xylene cyanole FF, $tRNA^{Arg}$, 5S Hela RNA, and $tRNA^{Arg}$ half molecules prepared with S1 nuclease (Harada and Dahlberg, 1975) were used as markers. Staining was carried out with "Stainsall" (Dingman and Peacock, 1971). For stripchart counting, the gels were counted in an Actigraph III thin-layer conveyor connected to a No. 8703 scaler and printer (all from Nuclear Chicago) operating at 1050 V, time constant 10 s, 0–5000 cpm range, at a chart speed of 60 cm/h, and scaler count interval of 30 s (Rushizky and Mozejko, 1975). Gels were run in duplicate or triplicate, and the recovery of total cpm from identical digests varied by less than 6%.

On a preparative scale, 100 mg of calf thymus DNA was hydrolyzed with various amounts of DNase at DNA concentrations of 0.04 and 1.2 mg/mL. The enzyme was inactivated by addition of EDTA to 5 mM and the digest fractionated by chromatography on DEAE-Sephadex A-25 columns, 2.0 × 72 cm, in 7 M urea–0.2 M NaCl–20 mM Tris-HCl, pH 7.3, with 6-L linear gradients to 0.5 M NaCl at a flow rate of 72 mL/h, 18 mL/fraction (Rushizky et al., 1964). In addition to being inactive in 7 M urea, the DNase is not bound on DEAE-Sephadex under these conditions. Before column chromatography, aliquots of digests were also examined by paper chromatography and electrophoresis (Roberts et al., 1962).

The material in the major peaks was pooled and desalted on DEAE-cellulose. After denaturation by boiling, chain lengths were determined by polyacrylamide gel electrophoresis as described above. To characterize the end groups of the compounds, they were digested with micrococcal nuclease (Sulkowski and Laskowski, 1966; Roberts et al., 1962), S1 nuclease (Vogt, 1973), or spleen phosphodiesterase after prior dephosphorylation (Razzell and Khorana, 1961).

The DNase was also tested for its ability to hydrolyze the deoxy derivatives of ApApCp, ApApTp, ApApGp, ApGpGp, and mixed hexanucleotides derived from micrococcal nuclease digests of calf thymus DNA (Rushizky et al., 1972). The extent of hydrolysis was measured by paper chromatography and electrophoresis (Roberts et al., 1962).

Results

Enzyme Purification. A DNase from commercial preparations of *Aspergillus oryzae* was purified about 1550-fold with a yield of 25% (Table I). The high isoelectric point of the enzyme, 9.2, facilitates its isolation by ion-exchange chromatography. Thus, the enzyme was first freed of much contaminating material by passage through DEAE-cellulose at pH 7.3 where it was not bound, followed by adsorption on and elution from CM-cellulose at the same pH with a salt gradient (Table I). The apparent increase in total enzyme activity after the DEAE-cellulose step may be due to the removal of an inhibitor(s) that interfered with enzyme activity and/or the enzymatic assay.

Physical Properties of the Enzyme. Gel filtration on Sephadex G-100 gave a single peak of DNase activity at the elution position of a globular protein of molecular weight 48 000. Electrophoresis in discontinuous sodium dodecyl sulfate–polyacrylamide gels yielded a single protein band of molecular weight 58 000. The estimated accuracy of these mol wt determinations is ±8% (gel filtration) and ±5% (gel electrophoresis). Following isoelectric focusing, a single peak of DNase activity was found in the fraction at pH 9.2 (Figure 1).

TABLE II: Effect of Various Additions on DNase Activity as Measured by Acid Solubility Assay.[a]

Changes in assay solution	Concn (mM)	Rel act. (%)
None		100
Omit $MgCl_2$		9
$MgCl_2$[b]	0.1	72
$CoCl_2$[b]	1.0	196
$CoCl_2$[b]	0.1	106
$ZnCl_2$[b]	1.0	7
$CaCl_2$[b]	1.0	5
$MnCl_2$[b]	1.0	68
EDTA, NH_4 salt[b]	1.0	3
Add Na-phosphate	10.0	5
Add NaCl	300	2
Heat 5 min at 95 °C		31
Add SH-EtOH	1	96
Denatured DNA[c]		45
MS2 RNA[c]		8

[a] The assay solution contained 1 mM $MgCl_2$, 0.04 M Tris-HCl, pH 8.2, and 40 μg/mL of T7 DNA. The increase in A_{260} of 0.5/mL obtained (0.5 unit of enzyme) was set to 100%. [b] For tests of the metal requirements of the DNase, the 1.0 mM $MgCl_2$ in the assay solution was replaced as indicated. [c] The native T7 DNA in the assay solution was replaced by 40 μg/mL of heat-denatured T7 DNA, or by MS2 RNA.

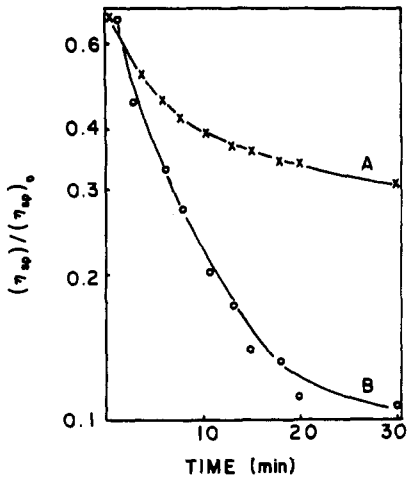

FIGURE 2: Effect of the *Aspergillus* DNase on the specific viscosity of DNA. (A) A Zimm low shear viscometer (Beckman) containing 3.1 mL of assay solution (40 μg of T7 DNA per mL, in 0.04 M Tris-HCl (pH 8.2)-1 mM $MgCl_2$ at 37 °C) was used. After the addition of 50 μL of DNase in water at 0.45 unit/mL, measurements of the time required for ten revolutions were taken. Changes in viscosity are represented as specific viscosity (η_{sp}) and are plotted as fractional values of the zero-time specific viscosity $(\eta_{sp})_0$. (B) As in A, but the digestion mixture also contained 0.1 mM $CoCl_2$.

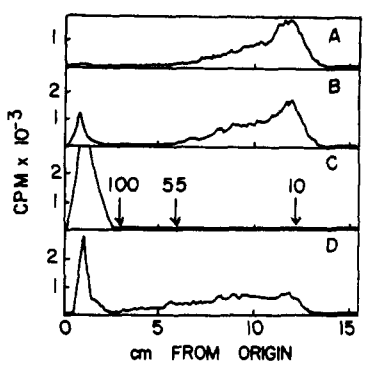

FIGURE 3: Fractionation of *Aspergillus* DNase digests of ^{32}P-labeled *E. coli* DNA by gel electrophoresis followed by strip chart counting. Chain length values are indicated by arrows. (A) Native DNA, hydrolyzed with 0.02 unit of enzyme per mg of substrate. (B) Heat-denatured DNA, hydrolyzed as in A. (C) Native DNA, hydrolyzed as in A, but in 1 mM EDTA. (D) Native DNA, hydrolyzed with 0.004 unit of enzyme per mg of substrate.

Catalytic Properties of the Enzyme. As measured by the generation of acid-soluble products, 0.25 unit of the *Aspergillus* DNase produced the same amount of hydrolysis of T7 DNA as 7.3 unit of DNase I. There was no production of acid-soluble fragments when the substrate concentration was raised from the usual 40 μg/mL to 250 or 1000 μg/mL. The pH optimum for DNase activity was at 8.2, with 50% of the maximum activity remaining at pH 9.2 and 6.7, respectively. In 40 mM sodium borate, pH 8.2, the enzyme activity was about 30% of that with Tris-Cl.

Table II shows the effect on enzyme activity of changes in the assay conditions. At the concentration tested, denatured DNA is a less effective substrate than native DNA and RNA is a poor substrate for the enzyme. Sodium phosphate (10 mM) and NaCl (0.3 M) inhibit enzyme activity. Heating to 95 °C for 5 min destroys two-thirds of the activity. The enzyme is active in the presence of 1 mM β-mercaptoethanol. Co^{2+}, Mg^{2+}, and Mn^{2+} activate the enzyme, while Ca^{2+} and Zn^{2+} do not; EDTA inhibits the activation by Mg^{2+}.

Co^{2+} appeared to be the most effective divalent cation in activating enzyme activity. This was confirmed by viscometry, where the rate of hydrolysis in the presence of 0.1 mM Co^{2+} plus 1 mM Mg^{2+} was double the rate in the presence of 1 mM Mg^{2+} alone (Figure 2).

Characterization of the Products of DNase Digestion. Figure 3 shows the effect of substrate concentration and conformation on DNase activity. Digestion of ^{32}P-labeled DNA at a ratio of 0.02 unit of enzyme per mg of DNA generates DNA fragments ranging in length from 10 to 55 nucleotides (Figure 3A); a similar pattern was obtained using heat-denatured DNA, but a part of the DNA remained unhydrolyzed (Figure 3B). Digestion at a ratio of 0.004 unit of enzyme per mg of DNA generates a broader peak of DNA fragments and also leaves a portion of the DNA unhydrolyzed (Figure 3D). There is no DNase activity in the presence of 1 mM EDTA (Figure 3C).

Preparative scale column chromatography was used to define conditions for exhaustive hydrolysis of DNA. Digestion of 100 mg of native calf thymus DNA (1.2 mg/mL) at a ratio of 0.06 unit of enzyme per mg of substrate generates two major peaks of material absorbing at 260 nm separated by column chromatography on DEAE-Sephadex in 7 M urea (Figure 4). Polyacrylamide gel electrophoresis indicated an average length of 10 nucleotides for fragments in the first peak and 100 nucleotides for those in the second peak, which also contained fragments longer than 200 bases that did not enter the gel. Twenty-nine percent of the material applied to the column was recovered in peak 1 and 52% in peak 2. We detected no DNA fragments less than 5 nucleotides in length when the digests were examined by paper electrophoresis and chromatography prior to chromatography on DEAE-Sephadex. The drop in the A_{260} elution pattern after peak 1 was reproducibly obtained when Mg^{2+} or Co^{2+} was used to activate the enzyme, provided the DNA concentration at digestion was over 1 mg/mL. At lower DNA concentrations, such as with the ^{32}P-labeled DNA

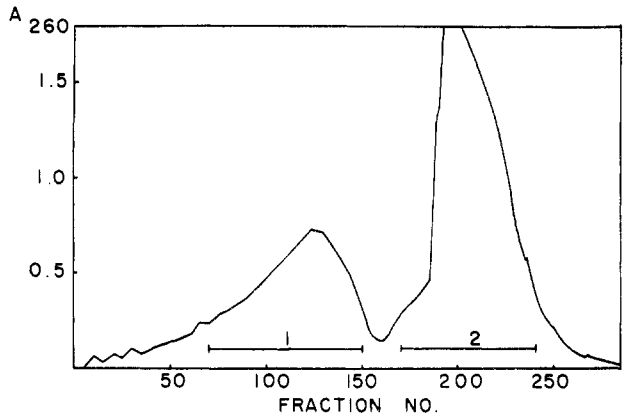

FIGURE 4: Column chromatography of *Aspergillus* DNase digests of 100 mg of calf thymus DNA on DEAE-Sephadex A-25 in 7 M urea–0.02 M Tris-HCl, pH 7.3, eluted with a linear gradient of NaCl from 0.2 to 0.5 M. The two major peaks are indicated.

fractionated by gel electrophoresis (Figures 3A, B, and D), no such separation between the two peaks was seen.

Under the above conditions, no further hydrolysis occurred after the repeated addition of more enzyme to a final ratio of 0.8 unit per mg of DNA. On the other hand, further hydrolysis was observed when the DNA concentration was decreased to 0.04 mg/mL. Under these conditions, the elution profile from DEAE-Sephadex showed a single broad peak with many shoulders, located in the range occupied by peaks 1 and 2 in Figure 4. However, there was no increase in the amount of short fragments eluted prior to peak 1.

The DNA fragments in peaks 1 and 2 of Figure 4 were further characterized after their hydrolysis to mononucleotides by other nucleases. Following digestion by S1 nuclease, no nucleosides were detectable by paper chromatography where, if present, 4% of the applied A_{260} could be detected. By contrast, about 11% of the material in peak 1 (Figure 4) was converted by micrococcal nuclease to nucleosides. These results indicated that at least 96% of the digestion products of the *Aspergillus* DNase were 5′, rather than 3′-terminal phosphate compounds. Following dephosphorylation and digestion with spleen phosphodiesterase, micrococcal nuclease, or S1 nuclease, 88% of the total A_{260} of nucleosides recovered from the 3′ end were adenosine, while at the 5′ end only guanosine and adenosine (molar ratio 2:3) were found.

The *Aspergillus* DNase did not hydrolyze tri- or hexanucleotides (generated by micrococcal nuclease digestion of calf thymus DNA) during incubation for 16 h at 37 °C at a ratio of 0.6 unit of enzyme per mg of oligomer (0.5 mg/mL). Furthermore, the DNase was free of phosphatase and nonspecific phosphodiesterase activity.

Discussion

The DNase isolated as described here was first noted in crude S1 nuclease preparations (Rushizky et al., 1975) because of its ability to hydrolyze double-stranded DNA. S1 nuclease has been described both as single-strand specific (Ando, 1966; Vogt, 1973; Beard et al., 1973) and as capable of hydrolyzing native DNA, albeit at a much slower rate (Godson, 1973; Johnson and St. John, 1974). Our results may explain these divergent reports. Because of the Zn^{2+} requirements and pH 4–5 optimum of S1 nuclease, the presence of the DNase described here would be hard to detect in S1 nuclease preparations. A convenient check on the possible presence of this *Aspergillus* DNase in S1 nuclease preparations would be passage through CM-cellulose at pH 7, a procedure which would not bind S1 nuclease.

The *Aspergillus* DNase itself is also difficult to assay in crude preparations. The original solution (Table I) contains a number of different DNases (Ando, 1966; Kato and Ikeda, 1968). However, the solution shows no activity as measured by the production of acid-soluble material. This implies the presence of inhibitors. During the pretreatment with DEAE- and CM-cellulose necessary to demonstrate activity in the original solution (Table I), a fraction of the DNase activity may be lost, giving actually a lower recovery than described here.

The inhibitors of DNase activity have not been identified. They are not enzyme-specific like those reported for *Bacillus amyloliquefaciens* RNase (Hartley and Smeaton, 1973) or for other *Aspergillus* enzymes (Uozumi et al., 1976), and may, in part, inhibit the assay itself. For example, a basic material elutes after the DNase during CM-cellulose chromatography that precipitates the DNA in the assay solution used to detect the nuclease. The basic material contains no detectable A_{280} absorption and no histones as revealed by gel electrophoresis. The insoluble DNA complex is dissociated by 2 M NaCl, but the DNase is inactive at such high salt concentrations.

Recovery of *Aspergillus* DNase activity from the same lot of crude amylase is also lowered by 25–50% when mercaptoethanol is omitted from buffer A, even though the purified enzyme is not activated by SH compounds. To detect the DNase by A_{280} absorption during CM-cellulose chromatography with a buffer of higher blank value due to mercaptoethanol requires purification of starting material on a scale as described.

A possible application for the *Aspergillus* DNase depends on its similarity to DNase I. The two enzymes resemble each other in several respects, such as activation by Co^{2+} and Mg^{2+} and production of compounds with 5′-terminal phosphate ends. Furthermore, both enzymes are subject to substrate inhibition and autoretardation (Laskowski, 1971). Thus, the rate of reaction slows down considerably in the presence of hydrolysis products; this is shown both by the low ability of the *Aspergillus* DNase to produce mononucleotides and short oligonucleotides when DNA at 1.2 mg/mL is digested on a preparative scale and by the generation of acid-soluble products only at low (40 μg/mL) DNA concentrations. The above product inhibition also takes precedence over the preference, if any, of the DNase to cleave DNAs next to certain bases. Thus, the *Aspergillus* enzyme hydrolyzes DNA so as to leave mostly purines at the ends of the oligomers formed, but does not cleave purine trinucleotides. In contrast to the above similarities between both enzymes, DNase I has a much lower isoelectric point at pH 5 rather than at 9.2. DNase I is one of several nucleases that have been used in studies of the structure of chromatin (Noll, 1974b; Simpson and Whitlock, 1976b; Weintraub and Groudine, 1976). Hydrolysis of the chromatin subunit with the *Aspergillus* DNase allows an evaluation of the influence of the physicochemical properties of both DNases on their interactions with nucleoprotein substrates (Whitlock et al., 1977).

Another possible application of the *Aspergillus* DNase may be as a tool in oligonucleotide chemistry. The enzyme discriminates against linkages of oligonucleotides shorter than 7–8, similar to the *E. coli* endonuclease I (Lehman et al., 1962). As described here the enzyme may, under certain conditions, be used to generate oligonucleotides of about ten bases in length. Such compounds are of interest in studying the interactions of DNA with histones in the chromatin subunit.

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