Purification and Characterization of an Acid Deoxyribonuclease from the Cultured Mycelia of *Cordyceps sinensis*

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A new acid deoxyribonuclease (DNase) was purified from the cultured mycelia of *Cordyceps sinensis*, and designated CSDNase. CSDNase was purified by (NH₄)₂SO₄ precipitation, Sephacryl S-100 HR gel filtration, weak anion-exchange HPLC, and gel filtration HPLC. The protein was single-chained, with an apparent molecular mass of ca. 34 kDa, as revealed by SDS-PAGE, and an isoelectric point of 7.05, as estimated by isoelectric focusing. CSDNase acted on both double-stranded (ds) and single-stranded (ss) DNA, but preferentially on dsDNA. The optimum pH of CSDNase was pH 5.5 and its optimum temperature 55°C. The activity of CSDNase was not dependent on divalent cations, but its enzymic activity was inhibited by high concentration of the cation: MgCl₂ above 150 mM, MnCl₂ above 200 mM, ZnCl₂ above 150 mM, CaCl₂ above 200 mM, NaCl above 300 mM, and KCl above 300 mM. CSDNase was found to hydrolyze DNA, and to generate 3-phosphate and 5-OH termini. These results indicate that the nucleolytic properties of CSDNase are essentially the same as those of other well-characterized acid DNases, and that CSDNase is a member of the acid DNase family. To our knowledge, this is the first report of an acid DNase in a fungus.

**Keywords:** Acid DNase, *Cordyceps sinensis*, Endonuclease

Introduction

Deoxyribonucleases (DNases) occur in various organisms, including fungi (Conneely *et al.*, 1976; Chen *et al.*, 1993), and digest DNAs, though they have different requirements for cofactors, cations, and optional pH levels. DNases may be broadly divided into two classes, DNase I (EC 3.1.21.1) and DNase II (EC 3.1.22.1), based upon their pH optima and metal ion dependencies (Cunningham and Laskowski, 1953). The DNase II class, also named the Acid DNases, is a group of enzymes that cleaves DNA in a double-stranded fashion, with an acidic pH optimum, and have no requirement for divalent metal ions (Bernard, 1971). This is in contrast to the DNase I class members, which cleave DNA by nicking at near neutral pHs, and which require divalent cations for full activity (Moore, 1981).

Acid DNases have been found in cells and are secreted by many organisms (Dulaney and Touster, 1972; Baker *et al.*, 1998). The earliest identification of an acid DNase was recorded in 1947 (Catcheside and Holmes, 1947), and acid DNases were biochemically characterized in the 1960s (Bernardi *et al.*, 1965). Acid DNases have been implicated in many cellular processes. Former research considered acid DNase as a lysosomal enzyme that can be reversibly associated with the lysosomal membrane. Recent work has implicated this enzyme with lysosomal diseases and cancer, and in the apoptotic process (Barry and Eastman, 1993). Now, most research on acid DNase is focused on mammalian acid DNase, though a few researchers have concentrated on insect acid DNases. However, comparatively little research is aimed at understanding plant and microorganism acid DNases, which also have the potential to contribute to our understanding of the structure and of the biological functions of these DNases. Over the past few years several DNases have been found in fungi, such as *Coprinus cinereus* (Lu *et al.*, 1988; Kitamura *et al.*, 1997), but no fungus acid DNA use has been characterized to date.

*Cordyceps sinensis* is a parasitic fungus that is of *Lepidoptera larvae*. For centuries, the fruiting bodies of *C. sinensis* have been used as both a food and a herbal tonic, named Dong-Chong-Xia-Cao in Mandarin China, and has been used to treat a variety of diseases. (SteinKraus and Whitfield, 1994; Zhu *et al.*, 1998a, 1998b). In this report, we...
describe the purification and characterization of a new acid DNase cultured from mycelia of C. Sinensis. We conducted a comprehensive characterization of this enzyme, and compared it with several established acid DNases from mammal, insect and protozoan. We found that the isolated CSDNase was in fact a member of the acid DNases. To our knowledge, this is the first report of an acid DNase in a fungus.

Materials and Methods

Materials  The Cordyceps sinensis (CCTCC AP99009) strain was obtained from China Center for Type Culture Collection (CCTCC), Sephacryl S-100 HR was purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). All HPLC columns were purchased from Agilent Technologies (Palo Alto, USA). Porcine spleen DNase II, Croatalus adamanteus venom phosphodiesterase I, and bovine spleen phosphodiesterase II were purchased from Sigma (St. Louis, USA). Protein molecular weight marker was from Fermentas (Burlington, Canada). Chemicals used routinely were of analytical grade and of the highest purity.  

Cultured Cordyceps sinensis  C. sinensis was first cultured on potato dextrose agar plates for 96 h at 28°C. These starter cultures were then transferred to nutrient broth containing (g/L): peptone 10, glucose 30, V_{2}, 0.05, KH2PO4 1, MgSO4 0.2, at pH6.0, on a rotary shaker at 120 rpm for 96 h at 28°C. The mycelia of C. sinensis were collected and stored at −80°C (Hsu et al., 2002).

Purification of CSDNase  Collected C. sinensis mycelia were fragmented using a Sonic Dismembrator in ice water, and extracted with extracting buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH7.0). After centrifugation, (NH4)2SO4 was added to the supernatant to 80% saturation and the mixture was allowed to stand for 4 h at 4°C. After centrifugation, (NH4)2SO4 was added to the supernatant to 80% saturation and the mixture was allowed to stand overnight at 4. The precipitate obtained using (NH4)2SO4 at 20–80% saturation was subsequently dialyzed and freeze-dried to give a crude powder. This crude protein powder was dissolved in buffer A (20 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH7.0), applied to a column of Sephacryl S-100 HR (2.6100 cm), and eluted with the same buffer. Fractions with DNase activity were dialyzed, concentrated, and then loaded on a SynChropak AX weak anion-exchange column (4.6 x 250 mm) in an Agilent 1100 HPLC system equilibrated with buffer B (20 mM Tris-HCl, 1 mM EDTA, pH7.5). Four adsorbed peaks were eluted using a linear concentration (0-0.5 M) gradient of NaCl in the same buffer. Fractions with DNase activity were concentrated and run on an Agilent GF-250 gel filtration column (9.6 x 250 mm) in an Agilent 1100 HPLC system; buffer A was used for column equilibration and elution. The active fraction was collected, dialyzed, concentrated, and used as the purified enzyme for subsequent experiments.

In addition, reverse-HPLC was used to test CSDNase purity. 20 µl of CSDNase solution was loaded on to an Agilent C18 column (4.6 x 250 mm), which was eluted with a linear concentration (10%-80%) gradient of acetonitrile in buffer B containing 0.05% trifluoroacetic acid at the flow rate of 1 ml/min. Absorption at 210 nm was monitored during the elution. All procedures were carried out at 4°C.

Assay for DNase activity  Acid DNase activity was measured by using a hyperchromicity assay, essentially as described by Kunitz (Kunitz, 1950) and Ito et al. (Ito et al., 1984). Briefly, enzyme solutions (10 µl) were added to a 1ml solution containing 40 µg of Calf thymus DNA in reaction buffer (50 mM ammonium acetate, 1 mM EDTA, pH5.5). After incubation 1 h at 55°C, the reaction was stopped by adding 1.0 ml of cold 5% perchloric acid to the mixture, which was stood for 10 min on ice, centrifuged at 10,000 x g for 15 min, and absorbance of the supernatant was measured at 260 nm. Enzyme activities were measured at two different enzyme concentrations at least. One unit of enzyme activity is defined as the amount of enzyme producing an optical density change of 0.001/min under the above conditions (Baker et al., 1998).

Alternately, the agarose gel electrophoresis method was used. Samples (5 µl) were added to a 45 µl solution containing 1 µg of different DNA substrates in reaction buffer. After incubation for 30 min at 55°C, the integrities of the DNA substrates were monitored by gel electrophoresis of the DNA in 0.8% (w/v) agarose gel and photographed under UV light (Kevin et al., 1998). In addition, DNase activity was monitored by plasmid nicking assay (Campbell and Jackson, 1980) using supercoiled plasmid pBR322 as a substrate.

SDS-PAGE and isoelectric focusing  SDS-PAGE was conducted according to the method of Laemmli and Faver (Laemmli and Faver, 1973). After electrophoresis, gels were stained with Coomassie Brilliant Blue. The molecular weight of CSDNase was then determined by comparing its electrophoretic mobility with those of molecular weight marker proteins.

Isoelectric focusing (IEF) was performed as described by Sun et al. (Sun et al., 2003). The isoelectric point was determined in 5% polyacrylamide gel containing 2% ampholine with a pH gradient from 3.5 to 9.5.

CSDNase activity gel electrophoresis  DNase activity by SDS-PAGE was performed according to the methods of Blank et al. (Blank et al., 1982). A 12% polyacrylamide gel containing 0.1 mg/ml of salmon testis DNA was used. Samples were electrophoresed without prior treatment with â-mercaptoethanol or boiling. After electrophoresis, the gels were rinsed with two changes of 25% isopropanol in 50 mM Tris-HCl (pH 7.0), followed by three changes of reaction buffer. All rinses were for 30 min at room temperature in 250 ml of the respective buffer. Rinsing was followed by 10 h of incubation at 55°C in reaction buffer. The incubated gels were then rinsed and stained with 0.5 µg/ml ethidium bromide for 30 min, the dark band produced by CSDNase was observed under ultraviolet light.

Activity of CSDNase on different DNA substrates  The activity of CSDNase on different DNA substrates was tested by the agarose gel electrophoresis. The purified CSDNase was incubated separately with different DNA substrates including ssDNA (Salmon testis DNA), dsDNA (calf thymus DNA, DNA), and supercoiled plasmid (pUC18, pBR322) in reaction buffer at 55°C.
Activity of CSDNase on RNA  Purified yeast tRNA was used as a substrate. CSDNase was incubated with 200 μg substrate in 150 μl of 50 mM ammonium acetate (pH 5.5) and then 350 μl of ice-cold 3.4% perchloric acid was added to terminate the reaction. After standing on ice for 15 min, the reaction mixture was centrifuged (12,000 x g 15 min) at 4°C. The OD260 of the supernatant was measured after suitable dilution (Deshpande et al., 2001). Ribonuclease activity was taken as defined previously (Wang and Ng, 2001).

Effect of pH and temperature on CSDNase activity and stability  The relative rates of DNA hydrolysis by CSDNase at various pH values were determined by hyperchromicity assay. Ammonium acetate buffer (50 mM) was used for pH 3-6, potassium phosphate buffer (50 mM) for pH 5-8, and Tris-HCl buffer (50 mM) for pH7-11. Enzyme stabilities were determined after pre-incubating CSDNase sample for 24 h at various pH levels without substrate.

The optimum temperature for the CSDNase activity was determined by hyperchromicity assay over the range 4-90°C. Thermal stability was determined by assaying the residual CSDNase activity after incubating CSDNase sample for 15 min at different temperature without substrate.

Activation of CSDNase by metal ions  The effect of metal ions on the activity of CSDNase was determined by hyperchromicity assay, and agarose gel electrophoresis was also used for detecting the effect of EDTA on the activity of CSDNase. In the test, the effect of divalent metal ions (MgCl2, MnCl2, ZnCl2, CaCl2), univalent metal ions (NaCl, KCl) and EDTA were examined on CSDNase activity.

Terminal phosphate position of the products generated by CSDNase digestion  The position of the terminal phosphate in the digestion products formed by CSDNase was determined using the method of Ikeda and Takata (2002). Calf thymus DNA was digested with CSDNase in reaction buffer at 55°C until approximately 25% of the DNA became acid-soluble. The reaction was stopped by heating at 80°C for 20 min. As a control, DNA cut by DNase I was prepared in the same manner, except that the reaction proceeded at 37°C in buffer consisting of 20 mM Tris-HCl (pH 7.5) and 5 mM MgCl2. To remove the terminal phosphate group, the DNA was treated with bacterial alkaline phosphatase at 37°C for 10 min before being reacted with phosphodiesterase.

Phosphodiesterase I from Crotalus adamanteus venom is a 3’-to-5’ exonuclease, and bovine spleen phosphodiesterase II is a 5’-to-3’ exonuclease. A limited digest (approx. 25% acid-soluble) of calf thymus DNA by CSDNase was used as substrate for phosphodiesterases I and II.

Results
Purification of CSDNase  A new DNase that acted at an acidic pH without divalent metal ions, was extracted from C. sinensis and designated CSDNase. The enzyme was purified by (NH4)2SO4 precipitation, gel filtration of Sephacryl S-100 HR, weak anion-exchange HPLC, and gel filtration HPLC. The yields and degrees of purification of the CSDNase present in the C. sinensis and the activity on calf thymus DNA are summarized in Table 1. Chromatographic elution profiles are shown in Fig. 1. From 20 g of C. sinensis mycelium (1 L culture medium of C. sinensis) approximately 0.5 mg of the purified CSDNase was obtained for analysis; enzyme purification was ca. 500 fold.

A single peak was observed when reverse-phase HPLC was used to test the purity of CSDNase (Fig. 2A). As shown in Figs. 2B, and 2D, the CSDNase sample obtained was also identified to be pure by SDS-PAGE and IEF.

Characterization of CSDNase  CSDNase was found to be a single-chain polypeptide, because it produced a single band by PAGE and SDS-PAGE (Fig. 2B 2C). The molecular weight of CSDNase in the native state was determined to be 36 kDa by gel-filtration HPLC using an Agilent GF250 column (Fig. 1C.). The purified CSDNase had an apparent molecular mass of 34 kDa by SDS-PAGE and by SDS activity gels using ethidium bromide (Fig. 2B). The difference between the molecular mass determined by SDS-PAGE and that determined by gel filtration may be due to a non-globular native protein shape. IEF showed that CSDNase had a pI of 7.05 (Fig. 2D).

Enzyme substrate specificity  As shown in Fig. 3, CSDNase acted on both dsDNA and ssDNA, but the enzyme attacked dsDNA at a 1.5 times higher rate than ssDNA (Fig. 4C). CSDNase also acted on DNA supercoiled plasmid dsDNA without selectivity (Fig. 3), but showed no RNase activity on yeast tRNA. These results indicate that the isolated CSDNase is an endonuclease that cleaves DNA as substrate.

Table 1. Purification of CSDNase

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Activity (units)</th>
<th>Protein (mg)</th>
<th>Specific activity (units/mg)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>2026.2</td>
<td>921</td>
<td>2.2</td>
<td>100</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ precipitation</td>
<td>1784.8</td>
<td>401.2</td>
<td>4.4</td>
<td>88.1</td>
</tr>
<tr>
<td>Sephacryl S-100 HR</td>
<td>1345.2</td>
<td>35.2</td>
<td>38.2</td>
<td>66.4</td>
</tr>
<tr>
<td>Weak anion-exchange HPLC</td>
<td>642.6</td>
<td>2.4</td>
<td>267.8</td>
<td>31.7</td>
</tr>
<tr>
<td>Gel filtration HPLC</td>
<td>598.6</td>
<td>0.5</td>
<td>997.2</td>
<td>24.6</td>
</tr>
</tbody>
</table>

Purification was initiated from 20 g dry cultured mycelia of Cordyceps sinensis.
Enzymatic properties of CSDNase

The optimum pH of CSDNase was pH 5.5 in 50 mM ammonium acetate buffer. At pHs of 4.5 and 7 the enzyme exhibited only a half of its maximum activity (Fig. 4A). In addition, the enzyme activity was unaffected after being incubated at pH 4-7 for 24 h in 37°C.

The enzyme was active at 20°C, and its activity increased as the incubation temperature increased up to an optimum temperature of 55°C (Fig. 4B). The enzyme was stable after incubation for 15 min at 65°C, but completely lost its activity after 15 min at 80°C.

The activity of CSDNase was not dependent on divalent or univalent cations, but was inhibited by high concentrations of cations: MgCl₂ above 150 mM, MnCl₂ above 200 mM, ZnCl₂ above 150 mM, CaCl₂ above 200 mM, NaCl above 300 mM, and KCl above 300 mM, and these conditions completely inhibited its activity. EDTA did not inhibit the activity of CSDNase even at 10 mM (Table 2), indicating that CSDNase does not require divalent cations for activity, and that it could be inhibited by high cation concentrations.

Mode of action of CSDNase

The hydrolytic effect of CSDNase on dsDNA was determined by examining it effect...
on plasmid DNA in the absence of bivalent metal ions at pH 5.5 and 37°C. Supercoiled plasmid DNA (form I) was digested with a small mount of CSDNase, and the products were subjected to agarose gel electrophoresis. The initial products generated by the enzyme initially were nicked plasmid DNAs (form II). Linear DNAs (form III) were then observed with an increase in nicked open circle DNA. Finally, all of the plasmid DNA was hydrolysed by the CSDNase (Fig. 3D 3E). Moreover, this hydrolysis pattern did not change when the DNA plasmid was changed. These results indicate that *C. sinensis* CSDNase is an endonuclease that causes double-strand breaks in DNA substrates.

**Fig. 3.** (A) Salmon testis DNA. Lane 1, no enzyme; Lane 2, with 0.05 unit CSDNase. (B) Calf thymus DNA; Lane 1, no enzyme; Lane 2: with 0.05 unit CSDNase. (C) λ DNA. Lane 1, no enzyme; Lane 2, with 0.05 unit CSDNase. (D) pBR322 plasmid DNA. Lane 1, no enzyme; Lane 2, with 0.05 unit CSDNase Lane 3, with 0.2 unit CSDNase. (E) pUC18 plasmid DNA. Lane 1, no enzyme; Lane 2, with 0.1 unit CSDNase Lane 3, with 0.4 unit CSDNase.

**Fig. 4.** (A) Effect of pH on the activity of CSDNase on Calf thymus DNA at 55°C ([ ]) Tris-HCl Buffer, [ ] ammonium acetate buffer, [ ] potassium phosphate buffer). (B) Effect of temperature on the activity of CSDNase on calf thymus DNA, in 50 mM ammonium acetate buffer (pH 5.5). (C) Hydrolysis of ssDNA and dsDNA by CSDNase for various times ([ ] ssDNA, [ ] dsDNA).

**Terminal phosphates of the hydrolysis products produced by CSDNase** The hydrolysis products formed by CSDNase were found to be readily cleaved by phosphodiesterase II, but not by phosphodiesterase I. After alkaline phosphatase treatment, the DNA products were readily cleaved by
phosphodiesterase I. In contrast, DNA products digested with DNase I (which produces 3'-OH and 5'-phosphate termini) were readily cleaved by phosphodiesterase I, but not by phosphodiesterase II. This finding indicates that the oligonucleotides produced had a phosphate group at the 3'-termini and a hydroxyl group at the 5'-termini, which is characteristic of acid DNases (Schomburg and Salzmann, 1991).

### Discussion

A new acid DNase, designated CSDNase, was isolated from the cultured mycelia of *Cordyceps sinensis*. CSDNase was purified by (NH₄)₂SO₄ precipitation and a series of chromatographic separations. The protein was found to be a heterodimeric protein, consisting of a 1:1 complex of the α and β polypeptide chains with molecular weights of 35 and 10 kDa, respectively (Liao, 1985; Liao et al., 1989). Another DNase II from the human liver was found to consist of three non-identical subunits (Taskeshita et al., 1998). Other reports suggest that DNase II enzymes from other sources consists of a single polypeptide chain with different molecular weights of approximately 25-40 kDa; e.g. 36-38 kDa from rat liver (Dulaney and Touster, 1972); 38 kDa from hog spleen (Bernardi, 1971); 32 kDa from human urine (Yasuda et al., 1992); 38 kDa from human gastric mucosa and cervix (Yamanaka et al., 1974); 26 kDa from bovine liver (Lesca, 1975); 45 kDa from human lymphoblasts (Harosh et al., 1991); *Euglena* acid DNase from *Euglena gracilis* SM-ZK (Ikeda and Takata, 2002), and 35 kDa enzyme with a DNase I-like structure exhibiting acidic DNase II-like activity from human (Appietto et al., 1997). The molecular mass of CSDNase was found to be ca. 34 kDa, which is in the reported range for a single-chained acid DNase. The isoelectric point of CSDNase is 7.05, which is similar to other acid DNases, e.g., DNase II from porcine spleen (pI 7.7), liver (pI 7.5), and gastric mucosa (pI 7.3) (Liao et al., 1989).

The catalytic properties of CSDNase are essentially the same as those of all other well described acid DNases, e.g., *Euglena* acid DNase (Ikeda and Takata, 2002), rat liver lysosomes acid DNase (Dulaney and Touster, 1972), porcine spleen acid DNase (Liao, 1985; Liao et al., 1989), human urine acid DNase (Yasuda et al., 1992), and human gastric mucosa and cervix uteri acid DNase (Yamanaka et al., 1974). CSDNase is an endonuclease, which is activated in low pH conditions, does not require divalent metal ions, is inhibited by high concentrations of cations, and is active against single and double stranded DNA. Like other acid DNases, CSDNase also acts preferentially on dsDNA. It is interesting to note that CSDNase showed nick activity on supercoiled plasmid dsDNA. As shown in Fig. 3D and 3E, the enzyme not only cleaved supercoiled plasmid DNA (form I) without bivalent metal ions at pH5.5 in 37°C, but also produced nicked supercoiled plasmid DNA (form II) on the reaction buffer, and lost its activity at high concentrations of cations, and is active against single and double stranded DNA. Like other acid DNases, CSDNase also acts preferentially on dsDNA and generates 3'-phosphate and 5'-OH termini. These findings indicate that CSDNase is a DNase II.

However, the activity of CSDNase differs slightly from other acid DNases. CSDNase has less preference for dsDNA than some other acid DNases. CSDNase hydrolyzes dsDNA about 1.5 times faster than ssDNA, but most acid DNases hydrolyze dsDNA about 3-4 times faster than ssDNA (Dulaney and Touster, 1972; Liao, 1985; Yasuda et al., 1992; Ikeda and Takata, 2002), and the acid DNase from human gastric mucosa and cervix uteri hydrolyze dsDNA about 10-20 times faster than ssDNA (Yamanaka et al., 1974). Moreover, CSDNase is inhibited only at high ion concentrations in the reaction buffer, and lost its activity at concentrations higher than 150-300 mM (Table 2), whereas most acid DNases lose their activities at ion concentrations >50M (Dulaney and Touster, 1972; Yamanaka et al., 1974; Liao, 1985; Yasuda et al., 1992; Ikeda and Takata, 2002). These differences may be the reason for the diversity of the individual enzyme’s action.

### Table 2. Effect of different concentrations of cations (at 55°C and pH 5.5) on the activity of CSDNase

<table>
<thead>
<tr>
<th>Salt</th>
<th>Relative activity (%)</th>
<th>Inhibiting concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>NaCl</td>
<td>105</td>
<td>106</td>
</tr>
<tr>
<td>KCl</td>
<td>102</td>
<td>101</td>
</tr>
<tr>
<td>MnCl₂</td>
<td>103</td>
<td>92</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>105</td>
<td>90</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>109</td>
<td>93</td>
</tr>
<tr>
<td>EDTA</td>
<td>98</td>
<td>85</td>
</tr>
</tbody>
</table>

In this experiment, the fungal origin of this DNase activity was confirmed by the finding that no DNase activity was found in the initial culture medium. Moreover, because almost no DNase activity could be detected in the culture medium, and the acid DNase activity of mycelia was no higher when mycelia were cultured after 96h, we can conclude that CSDNase is an endocellular enzyme.

Although acid DNase was first studied biochemically in the 1960's (Bernardi et al., 1965), their structures have not been elucidated and there is some debate concerning the structure of DNase II. Several reports suggest that proline spleen DNase II is a heterodimeric protein, consisting of a 1:1 complex of the α and β polypeptide chains with molecular weights of 35 and 10 kDa, respectively (Liao, 1985; Liao et al., 1989). Another DNase II from the human liver was found to consist of three non-identical subunits (Taskeshita et al., 1998). Other reports suggest that DNase II enzymes from other sources consists of a single polypeptide chain with different molecular weights of approximately 25-40 kDa; e.g. 36-38 kDa from rat liver (Dulaney and Touster, 1972); 38 kDa from hog spleen (Bernardi, 1971); 32 kDa from human urine (Yasuda et al., 1992); 38 kDa from human gastric mucosa and cervix (Yamanaka et al., 1974); 26 kDa from bovine liver (Lesca, 1975); 45 kDa from human lymphoblasts (Harosh et al., 1991); *Euglena* acid DNase from *Euglena gracilis* SM-ZK (Ikeda and Takata, 2002), and 35 kDa enzyme with a DNase I-like structure exhibiting acidic DNase II-like activity from human (Appietto et al., 1997). The molecular mass of CSDNase was found to be ca. 34 kDa, which is in the reported range for a single-chained acid DNase. The isoelectric point of CSDNase is 7.05, which is similar to other acid DNases, e.g., DNase II from porcine spleen (pI 7.7), liver (pI 7.5), and gastric mucosa (pI 7.3) (Liao et al., 1989).
enzyme that cleaves DNA in a mechanistically different manner from the DNA nicking activity of pancreatic DNase I. Using the same technique, scission by the DNase II isolated from rat small intestinal mucosa was shown to consist mainly of double-strand breaks (Anai et al., 1983), and a recent report showed that an acid DNase from Euglena gracilis also did not have nick activity on supercoiled plasmid DNA and could only break supercoiled plasmid DNA (Ikeda and Takata, 2002). But most recent reports have suggested that some acid DNases possess nicking activity (Harosh et al., 1991; Baker et al., 1998), which cleaves supercoiled plasmid DNA like DNase I, which nicks supercoiled plasmid DNA in the present of divalent cations. The reason for this different acid DNase activity on supercoiled plasmid dsDNA has not been clarified. We found that CSDNase has nick activity on supercoiled plasmid DNA, which indicates that CSDNase belongs to the subgroup of the acid DNase family with nicking activity.

The functions of CSDNase are unclear, but considering the fact that its activity is primarily expressed during fungal mycelium growth (data not showed) and it is an endocellular enzyme, one might conjecture that CSDNase may be involved in the meiosis of the fungal mycelium. Further works are in progress to determine the amino acid sequence and the functions of this enzyme.

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