Active-site characterization of S1 nuclease I

Affinity purification and influence of amino-group modification

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A simple procedure, involving heat-treatment, DEAE-Sephadex, AMP-Sepharose and Bio-Gel P-60 chromatography, was developed for the purification of S1 nuclease to homogeneity from commercially available Takadiastase powder. Chemical modification of the amino groups of purified S1 nuclease revealed that lysine is essential for single-stranded DNAase, RNAase and phosphomonoesterase activities associated with the enzyme. The kinetics of inactivation suggested the involvement of a single lysine residue in the active site of the enzyme. Additionally, lysine modification was accompanied by a concomitant loss of all the activities of the enzyme, indicating the presence of a common catalytic site responsible for the hydrolysis of single-stranded DNA, RNA and 3'-AMP. Substrate-protection and inhibitor-binding studies on enzyme modified with 2,4,6-trinitrobenzenesulphonic acid showed that lysine may be involved in the substrate binding.

INTRODUCTION

Single-strand-specific nuclease from *Aspergillus oryzae* (S1 nuclease, EC 3.1.30.1) is a multifunctional enzyme which acts on single-stranded (ss)DNA, RNA and 3'-AMP (Oleson & Sasakuma, 1980). Although this enzyme has been purified (Vogt, 1973; Oleson & Sasakuma, 1980; Shishido & Habuka, 1986) and extensively used as an analytical tool for the characterization of nucleic acid structure (Rushizky, 1981), very little information is available regarding its molecular enzymology. Through competitive inhibition studies, Oleson & Hoganson (1981) showed that the hydrolysis of polynucleotides and mononucleotides is carried out by a common catalytic site. A similar observation was also made by Reddy & Shankar (1989) while studying the immobilization of S1 nuclease on concanavalin A-Sepharose. However, no reports are available till now regarding the active-site nature of S1 nuclease. In the case of enzyme(s) acting on anionic substrates, lysine and/or arginine has been implicated in substrate binding (Riordan, 1979; Richardson *et al.*, 1990). Moreover, lysine residues have also been implicated in the binding of ssDNA to ssDNA-binding proteins (Anderson *et al.*, 1975; Bandyopadhyay & Cheng-Wen, 1978). Since S1 nuclease is also an ssDNA-binding protein acting on anionic substrates, chemical modification of amino groups was carried out to assess their role in the catalytic activity of the enzyme.

MATERIALS AND METHODS

Materials

Materials were obtained as indicated: Takadiastase (Uni-Sankyo, Hyderabad, India); DEAE-Sephadex A-50 and AH-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden); Bio-Gel P-60 (Bio-Rad, Richmond, CA, U.S.A.); RNA (Sisco Research Laboratories, Bombay, India); sodium borohydride and formaldehyde (BDH, Bombay, India); 3'-AMP, 5'-AMP, dATP, 2,4,6-trinitrobenzenesulphonic acid (TNBS) and citraconic anhydride (Sigma Chemical Co., St. Louis, MO, U.S.A.); perchloric acid (Qualigens, Bombay, India); uranyl acetate (Loba

Chemie Indoaustrian Co., Bombay, India). [α - 32 P]dATP was obtained from BARC, Bombay, India. All other chemicals used were of analytical grade.

High- M_r DNA from buffalo liver was isolated according to the method of Mehra & Ranjekar (1979). 5'-AMP-Sepharose was prepared as described by Uchida & Schibata (1981). *p*-Nitrophenylglyoxal was prepared from *p*-nitroacetophenone according to the method of Steinbach & Becker (1954).

Enzyme assays

ssDNAase activity of S1 nuclease. The standard reaction mixture of 1 ml contained 50 μ g of sonicated and heat-denatured buffalo liver DNA, 1 mM-ZnSO₄, 50 mM-NaCl and 5% (v/v) glycerol in 30 mM-sodium acetate buffer, pH 4.6, and appropriately diluted enzyme. The reaction was initiated by the addition of the enzyme followed by incubation at 37 °C for 15 min. The reaction was then terminated by the addition of 1 ml of 10% (v/v) chilled perchloric acid and 1 ml of 0.2% (w/v) BSA. The mixture was left on ice for 10 min and then centrifuged (800 g, 10 min) to sediment the precipitate. The acid-soluble nucleotides in the supernatant were measured at 260 nm. The acid-soluble deoxyribonucleotides were estimated by assuming a molar absorption coefficient of 10000 litre·mol⁻¹·cm⁻¹ (Curtis *et al.*, 1966).

RNAase activity of S1 nuclease. RNA-degrading activity of the enzyme was determined according to the method of Uchida & Egami (1967b) with slight modifications. The standard reaction mixture of 0.75 ml contained 3 mg of yeast RNA, 1 mM-ZnSO₄, 50 mM-NaCl and 5% (v/v) glycerol in 30 mM-sodium acetate buffer, pH 4.6, and appropriately diluted enzyme. The reaction was initiated by the addition of RNA followed by incubation at 37 °C for 15 min. The reaction was then arrested by the addition of 0.25 ml of chilled uranyl reagent [0.75% (w/v) uranyl acetate in 25% (v/v) perchloric acid] and the resulting precipitate was immediately removed by centrifugation (800 g, 10 min). Subsequently, 0.1 ml of the supernatant was diluted with 2.5 ml of distilled water and the acid-soluble nucleotides were measured at 260 nm. The amount of acid-soluble ribonucleotides was calcu-

Abbreviations used: ssDNA, single-stranded DNA; TNBS, 2,4,6-trinitrobenzenesulphonic acid.

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Table 1. Purification of S1 nuclease

Step	Units	Protein (A_{280})	Specific activity (units/ A_{280})	Fold purification	Recovery (%)
Crude	25000	1540	16.23	1.00	100
Heat-treatment	24000	1450	16.55	1.02	96.0
DEAE-Sephadex chromatography	12000	109	110.10	6.78	48.0
5'-AMP-Sepharose chromatography	10000	14	714.30	44.00	40.0
Bio-Gel P-60 chromatography	6800	5	1360	83.79	27.2

Fig. 1. Electrophoresis of purified S1 nuclease

(a) 10% (w/v) polyacrylamide gel, Tris/glycine buffer, pH 8.3, current 4 mA/tube, protein loaded 75 μ g; (b) SDS/polyacrylamide gel (10%, w/v), Tris/glycine buffer, pH 8.3, SDS 0.1%, current 6 mA/tube, protein loaded 75 μ g; (c) isoelectric focusing in 10% (w/v) polyacrylamide gel, pH range 3.0–10.0, protein loaded 50 μ g.

lated by assuming a molar absorption coefficient of 10600 litre $\cdot$ mol⁻¹ $\cdot$ cm⁻¹ (Curtis *et al.*, 1966).

One unit of ssDNAase or RNAase activity is defined as the amount of enzyme required to liberate 1 μ mol of acid-soluble nucleotides/min under the assay conditions.

Phosphomonoesterase activity of S1 nuclease. This was carried out using 3'-AMP as the substrate. The total reaction mixture of 2 ml contained 1 mM-3'-AMP, 1 mM-ZnSO₄, 50 mM-NaCl and 5% (v/v) glycerol in 30 mM-sodium acetate buffer, pH 4.6, and appropriately diluted enzyme. After incubation at 37 °C for 15 min, the reaction was terminated by the addition of 2 ml of freshly prepared Chen's reagent (Chen *et al.*, 1956). The blue

colour that developed after 30 min incubation at 37 °C was read at 660 nm.

One unit of phosphomonoesterase activity is defined as the amount of enzyme required to liberate 1 μ mol of inorganic phosphate/min under the assay conditions.

Assay of RNAase T1 and RNAase T2. These were carried out according to methods of Uchida & Egami (1967a, b).

Protein determination

During the enzyme-purification steps, protein concentrations were monitored by assuming a relationship $A_{cm, 280}^{1\%} = 10$. However, for chemical-modification studies, protein concentration

Fig. 2. Effect of TNBS concentration on the activities of purified S1 nuclease

○, ssDNAase; ▲, RNAase; ×, phosphomonoesterase; ■, number of NH₂ groups modified.

was determined by the method of Lowry *et al.* (1951), with BSA as standard.

Purification of S1 nuclease

Unless otherwise stated, all the operations were carried out at 4 °C. During the purification steps, S1 nuclease activity was monitored using sonicated and heat-denatured DNA.

Heat-treatment. Takadiastase powder (2 g) was suspended in 50 ml of buffer A [300 mM-sodium acetate buffer, pH 5.5, containing 1 mM-ZnSO₄, 50 mM-NaCl and 5% (v/v) glycerol] and stirred for 1 h. The insoluble material was removed by centrifugation (6800 *g* for 10 min). The supernatant was then heated at 70 °C for 90 s with vigorous swirling, chilled immediately on ice and used for DEAE-Sephadex chromatography.

DEAE-Sephadex A-50 chromatography. The heat-treated enzyme sample was adsorbed on a DEAE-Sephadex column (2 cm × 20 cm) equilibrated at pH 5.5 with buffer B [50 mM-sodium acetate buffer containing 1 mM-ZnSO₄, 50 mM-NaCl and 5% (v/v) glycerol] at a flow rate of 16 ml/h. The column was then washed with buffer B, till the flow-through fractions showed no nuclease activity. Subsequently, the elution of the bound enzyme was affected with a linear gradient, 400 ml total volume, of NaCl (0–400 mM) in buffer B. Fractions of volume 4 ml were collected at a rate of 16 ml/h and fractions having specific activity greater than 100 units/mg were pooled and dialysed extensively against buffer B and used for the affinity step.

Affinity chromatography on 5'-AMP-Sepharose. The dialysed enzyme was then adsorbed on an AMP-Sepharose column (2 cm × 8 cm), pre-equilibrated at pH 5.5 with buffer B, at a rate of 12 ml/h. The column was washed with the same buffer, until the effluent showed no nuclease activity. Subsequently, the bound enzyme was eluted with a linear gradient, 300 ml total volume, of NaCl (0–500 mM) in buffer B. Fractions (3 ml) were collected at a rate of 12 ml/h and those having specific activity greater than 500 units/mg were pooled and concentrated to 4 ml using an Amicon ultrafiltration unit fitted with a PM10 membrane.

Gel filtration on Bio-Gel P-60. Concentrated enzyme sample (2 ml) was chromatographed on a Bio-Gel P-60 column (2 cm × 125 cm) equilibrated at pH 5.0 with buffer C [30 mM-sodium acetate buffer containing 1 mM-ZnSO₄, 50 mM-NaCl and 5% (v/v) glycerol] at a rate of 8 ml/h. Fractions (2 ml) were collected and those having specific activity greater than 1200 units/mg were pooled, concentrated and stored at –20 °C until further use. No loss of activity was observed when the purified enzyme was stored under these conditions.

Electrophoresis

Native and SDS/PAGE were carried out at pH 8.3 according to the method of Laemmli (1970). After electrophoresis, the native gels were stained with Coomassie Brilliant Blue G-250 (Blakesley & Boezi, 1977) whereas the SDS-containing gels were stained with Coomassie Brilliant Blue R-250 and destained using methanol/acetic acid/water (5:2:13, by vol.). Isoelectric focusing in polyacrylamide gels was carried as described by Vesterberg (1972) over the pH range 3.0–10.0.

M_r determination

M_r determination was carried out on Bio-Gel P-60 using ovalbumin (45000), soya-bean trypsin inhibitor (20100), myoglobin (17000) and lysozyme (14300) as reference proteins. *M_r* was also determined using SDS/10% (w/v) polyacrylamide gels (pH 8.3) as described by Laemmli (1970) with BSA (66000), ovalbumin (45000), carbonic anhydrase (29000) and lysozyme (14300) as reference proteins.

Chemical-modification studies

In chemical-modification studies, the residual activity of the modified enzyme was determined with all three substrates, namely ssDNA, RNA and 3'-AMP.

Reaction with TNBS. The total reaction mixture of 2 ml, containing 0.5 ml of S1 nuclease (250 µg) and 1 ml of 4% (w/v) NaHCO₃, was incubated with various concentrations of TNBS (0.1–0.5 mM) at 37 °C, in the dark. Samples were withdrawn at suitable intervals and the reaction was terminated by adjusting the pH to 4.6. Subsequently, the residual activities were determined under standard assay conditions. Enzyme samples incubated in the absence of TNBS served as control. The number of amino groups modified was determined spectrophotometrically by assuming a molar absorption coefficient of 9950 litre·mol^{–1}·cm^{–1} for the trinitrophenylated lysine (Habeeb, 1966).

Substrate-protection studies. The effect of substrate protection was studied by preincubating the enzyme with an excess amount of ssDNA, RNA and 3'-AMP for 20 min, followed by TNBS treatment.

Inhibitor-binding studies. TNBS (0.5 mM)-modified S1 nuclease (1 nmol) in 30 mM-sodium acetate buffer, pH 4.6 (containing 1 mM-ZnSO₄, 50 mM-NaCl and 5% glycerol) was mixed with 1 nmol of dATP. A small amount of [α-³²P]dATP was added to monitor the binding. The reaction mixture was then incubated at room temperature for 20 min followed by gel filtration on a Bio-Gel P-10 column (0.5 cm × 5 cm) equilibrated with the same buffer. Fractions of volume 0.3 ml were collected at a rate of 0.3 ml/min and radioactivity was determined on a Beckman liquid-scintillation counter. Unmodified enzyme incubated under identical conditions served as control.

Citraconylation. The amino groups of S1 nuclease were reversibly blocked with citraconic anhydride according to the method of Dixon & Perham (1968). Citraconic anhydride was diluted with dioxane and the concentration of the diluted reagent was 228 mM. Dioxane at this concentration did not have any adverse effect on the activity of S1 nuclease. Purified S1 nuclease (1 mg)

Fig. 3. Pseudo first-order plots for the inactivation of S1 nuclease by TNBS

(a) ssDNAase; (b) RNAase; (c) phosphomonoesterase. Concentrations of TNBS were 0 mM (●), 0.1 mM (▲), 0.2 mM (×), 0.3 mM (○) and 0.5 mM (■). Insets: second-order plots of the pseudo-first-order rate constants ($k_{app.}$) (min^{-1}) of inactivation at different concentrations of TNBS.

in 7 ml of 100 mM- NaHCO_3 buffer, pH 8.0, was treated at room temperature with a total of 310 μl of citraconic anhydride. The reagent was added in six instalments and the pH of the reaction mixture was maintained at 8.0 by addition of 1 M-NaOH. After every addition, a portion was removed and assayed for enzyme activities and number of free amino groups. The control consisted of enzyme samples incubated under identical conditions without citraconic anhydride. The total number of amino groups in native and modified enzyme samples were estimated by the TNBS method of Habeeb (1966). Decitraconylation was achieved by incubating the modified enzyme samples at pH 4.0 and 4 °C for 15 h, followed by assay of the enzyme activities.

Reductive methylation. This was carried out essentially as described by Means & Feeney (1968). To 1 ml of purified S1 nuclease (200 μg) in 200 mM-sodium borate buffer (pH 9.0) at 0 °C, 0.1 ml of sodium borohydride (0.5 mg/ml) was added followed by the addition of six samples (20 μl each) of 0.35% (v/v) formaldehyde solution, at an interval of 10 min. At the end of the reaction, the residual activities and the number of amino groups modified were determined as described previously. Enzyme incubated in the absence of formaldehyde served as control.

Reaction with *p*-nitrophenylglyoxal. S1 nuclease (500 μg) in 50 mM-Hepes buffer, pH 8.0, containing 150 mM-sodium ascorbate, was incubated with 25 μl of 10% *p*-nitrophenylglyoxal in methanol at 30 °C for 30 min. Subsequently, the residual activities were measured under standard assay conditions. Enzyme sample incubated in the absence of *p*-nitrophenylglyoxal served as control. The number of arginine residues modified was determined according to the method of Yamasaki *et al.* (1981).

RESULTS

Purification of S1 nuclease

The results of a typical procedure for the purification of S1 nuclease to homogeneity are summarized in Table 1. The enzyme was purified approximately 84-fold with an overall yield of 27%. S1 nuclease obtained after DEAE-Sephadex chromatography, although it showed high specific activity, contained a significant amount of RNAase activity. Subsequently, the RNAase activity was identified as that of RNAase T1, on the basis of its optimum pH, heat-stability at pH 2.0 and non-glycoprotein nature. Our efforts to separate S1 nuclease from RNAase T1 on 5'-AMP-Sepharose failed, as the latter not only bound to the matrix but was also co-eluted with S1 nuclease. However, this step helped in removing the coloured impurities and non-nucleotide-binding proteins. Finally, chromatography on Bio-Gel P-60 yielded a homogeneous S1 nuclease preparation. The purified enzyme moved as a single band in native, SDS and isoelectrofocusing gels, indicating its homogeneity (Fig. 1). The M_r of the pure enzyme determined by gel filtration and SDS/PAGE was 32000 and 32360 respectively. The pI of the purified enzyme was 4.0.

Chemical-modification studies

Purified S1 nuclease, when incubated with 0.5 mM-TNBS at pH 8.0 for 30 min, lost 65–70% of its activity towards ssDNA, RNA and 3'-AMP. The inactivation was dependent on the concentration of the reagent and the number of amino groups modified (Fig. 2). However, no loss of activity was observed in control samples. The logarithm of residual activity plotted as a

Table 2. Influence of lysine modification on the activity of S1 nuclease

For experimental details see the Materials and methods section.

Incubation mixture	Residual activity (%)	dATP binding (%)
Enzyme	100	100
Enzyme + TNBS	33	32
Enzyme + ssDNA (1 mg) + TNBS	65	—
Enzyme + RNA (1 mg) + TNBS	62	—
Enzyme + 3'-AMP (1 mM) + TNBS	65	—

Fig. 4. Citraconylation of S1 nuclease

(a) Influence of citraconic anhydride concentration on the activities of purified S1 nuclease. (b) Changes in the activities of S1 nuclease as a function of modified NH₂ groups. ●, ssDNAase; ○, RNAase; ×, phosphomonoesterase.

Table 3. Effect of citraconylation and reductive methylation on the activity of S1 nuclease

Modification reaction	Lysine residues modified	Residual activity (%)		
		ssDNAase	RNAase	AMPase*
Control	0	100	100	100
Citraconylation	1.0	55	60	60
Decitraconylation	0.3	75	70	70
Reductive methylation	1.2	40	40	40

* Phosphomonoesterase activity.

function of time at various TNBS concentrations was linear up to 33, 28 and 34% of the initial activity towards ssDNA, RNA and 3'-AMP respectively. TNBS-mediated inactivation followed pseudo-first-order kinetics at any fixed concentration of the reagent. The pseudo-first-order rate constants were calculated from the slope of plots of log (percentage residual activity) versus reaction time and the order was determined from the slope of the plots of log ($k_{app.}$) against log [TNBS]. These plots (insets of Fig. 3) indicated that the loss of enzyme activity towards all the three

substrates occurred as a result of modification of a single lysine residue per molecule of the enzyme.

TNBS-mediated inactivation of S1 nuclease could be prevented to a great extent, by incubating the enzyme with excess ssDNA, RNA and 3'-AMP before the modification reaction. In addition, the TNBS-modified enzyme showed a significant decrease in its ability to bind dATP, a potent competitive inhibitor of S1 nuclease (Table 2).

Citraconylation of S1 nuclease resulted in 70–75% loss in its initial activity and the inactivation was concentration-dependent (Fig. 4a). The plot of percentage residual activity against the number of amino groups modified revealed that the loss of enzyme activity towards ssDNA, RNA and 3'-AMP resulted from the modification of one lysine residue (Fig. 4b). Decitraconylation of the enzyme restored 70–75% of its initial activity, suggesting the involvement of lysine in the catalytic activity of S1 nuclease. Further, reductive methylation of the enzyme resulted in 60% loss in its initial activity towards all the substrates. Estimation of free amino groups after modification showed that the loss of activity was due to the modification of one lysine residue (Table 3).

Modification of arginine residues by treating the enzyme with *p*-nitrophenylglyoxal, although it resulted in the modification of two arginine residues out of a total of three (Shishido & Habuka, 1986), did not have any effect on the activity of purified S1 nuclease, indicating that arginine residues may not have any role in the catalytic activity of the enzyme.

DISCUSSION

Since a large amount of the enzyme is required for structural studies, initially attempts were made to develop a simple procedure to obtain a homogeneous enzyme with good recovery. In the present studies, DEAE-Sephadex chromatography was carried out at pH 5.5, as under this condition it is possible to separate RNAase T2 completely from S1 nuclease. Complete removal of RNAase T2 was essential at this step, as our previous experience showed that removal of trace amounts of RNAase T2 presented considerable difficulties because of their similar physicochemical properties, such as M_r , glycoprotein nature and nucleotide binding (Uchida & Egami, 1971; Rushizky, 1981). However, the pooled S1 nuclease fractions obtained after DEAE-Sephadex chromatography contained a significant amount of RNAase T1 activity. Similar observations were also made by Ando (1966) and Vogt (1973) while purifying S1 nuclease on DEAE-cellulose at pH 7.0–7.5. For further purification, affinity chromatography on 5'-AMP-Sepharose was chosen, as 5'-AMP is a competitive inhibitor of S1 nuclease having $K_i = 2.43 \times 10^{-4}$ M (Oleson & Hoganson, 1981) and thus has desirable properties as an affinity ligand. The inability of 5'-AMP-Sepharose to separate S1 nuclease and RNAase T1 can be correlated to the comparable affinities of these enzymes to the bound nucleotide. In fact, RNAase T1 has been purified on several nucleotide-affinity matrices (Takahashi & Moore, 1982). Nevertheless, a considerable degree of purification of S1 nuclease could be achieved by this step, in addition to removal of coloured impurities. Although Rushizky *et al.* (1975) separated RNAase T1 and S1 nuclease on DEAE-cellulose at pH 5.0, we preferred to chromatograph the enzyme on Bio-Gel P-60, as it is possible to remove RNAase T1 (M_r 11000) from S1 nuclease (M_r 32000) because of the vast difference in their M_r values. This procedure yielded an enzyme preparation which was electrophoretically homogeneous (Fig. 1). The purified enzyme is a single polypeptide chain with an M_r of 32000 having a pI of 4.0. Our values are consistent with that of Shishido & Habuka (1986). Oleson & Sasakuma (1980) purified S1 nuclease to near-homogeneity (97–99% pure) in

seven steps with 32% recovery. Subsequently, Shishido & Habuka (1986), starting from 30 mg of a partially purified S1 nuclease preparation, obtained 8 mg of pure enzyme in three steps. In comparison, our method offers a simple and rapid procedure for the purification of S1 nuclease to homogeneity with comparable yields.

It has been reported that lysine and arginine residues have an essential role in the active site of enzymes binding to anionic substrates (Riordan, 1979; Richardson *et al.*, 1990). The anionic nature of the substrates of S1 nuclease prompted us to look for the possible involvement of amino groups in the catalytic activity of the enzyme. When lysine residues of the enzyme were modified by TNBS, it lost 65–70% of its activity towards ssDNA, RNA and 3'-AMP, indicating that lysine may have a role in the catalytic activity of S1 nuclease (Fig. 2). Kinetic analysis of TNBS inactivation revealed that the loss of activity towards all the substrates is due to the modification of a single lysine residue (Fig. 3). The involvement of lysine residues in the catalytic activity of S1 nuclease was further ascertained by acylation and reductive methylation. Citraconylation of the purified enzyme led to its rapid inactivation and 70–75% of its initial activity was lost towards ssDNA, RNA and 3'-AMP (Fig. 4a). Decitraconylation could recover a significant amount of its activity towards all the substrates. Similar results were obtained when lysine residues of S1 nuclease were converted into *NN'*-dimethyl-lysine by reductive methylation. The loss of activity on citraconylation and reductive methylation resulted from the modification of one lysine residue per molecule of the enzyme (Table 3). The above results point towards the presence of lysine at or near the active site of S1 nuclease. Moreover, the comparable loss of activities of S1 nuclease as a result of lysine modification by different reagents suggests that modification does not result in a drastic change in the active-site conformation of the enzyme.

Substrate-protection studies on TNBS-modified enzyme revealed that the inactivation could be prevented to a considerable extent by preincubating the enzyme with excess ssDNA, RNA and 3'-AMP. Additionally, the modified enzyme showed decreased dATP binding (Table 2). These results indicate that lysine may be involved in the substrate binding. In the case of RNAase A, it has been shown that lysine residues are involved in the substrate binding (Richardson *et al.*, 1990).

As mentioned earlier, S1 nuclease catalyses the hydrolysis of ssDNA, RNA and 3'-AMP (Oleson & Sasakuma, 1980). Oleson & Hoganson (1981) interpreted the ability of 3'-AMP to inhibit the hydrolysis of ssDNA competitively and vice versa to the existence of a common catalytic site for the hydrolysis of both types of substrate. In the present studies, the parallel loss of all three activities of S1 nuclease on lysine modification coupled with substrate-protection and decitraconylation data strongly suggest the presence of a common catalytic site for the hydrolysis

of both monomeric and polymeric substrates. Our results are consistent with those of Oleson & Hoganson (1981) and Reddy & Shankar (1989).

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