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Review

Single-strand-specific nuclease from *Basidiobolus haptosporus* (Nuclease Bh1)

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Nuclease Bh1 is a multifunctional enzyme and catalyses the hydrolysis of ssDNA, 3'AMP and RNA in a ratio of approximately 1:1:0.05. It is a single polypeptide chain of 30 kDa and contains 15% carbohydrate. All three activities of the enzyme showed the same pI, indicating that they are associated with the same protein. The ssDNase, 3'AMPase and RNase activities of nuclease Bh1 showed the same pH and temperature optima and stability. Nuclease Bh1 neither showed an obligate requirement metal ion for its activity nor the activity was stimulated in presence of metal ions but it is a zinc metalloprotein. Moreover, they exhibited similar sensitivity towards divalent cations, metal chelators, thiol reagents, protein denaturants and guanosine 5' nucleotides. It hydrolyzed DNA endonucleolytically with a preference for guanylic acid linkages whereas; it showed an endo-exo mode of action on RNA, with a preference for adenylic acid linkages. Cytidylic acid linkages were resistant to cleavage. The phosphormonoesterase activity of the enzyme is 3' ribonucleotide specific. Active site nature suggested the involvement of lysine in substrate binding and carboxylate in catalysis.

Key words: *Basidiobolus haptosporus,* single-strand-specific nuclease, nuclease Bh1, multifunctional enzyme, characteristics.

INTRODUCTION

Single-strand-specific nucleases are wide spread in distribution. Their ability to recognize a wide variety of nucleic acid structures has lead to their extensive application as probes for the determination of nucleic acid structure (Desai and Shankar, 2003). Most of the analytically important enzymes share common properties such as multiple activity, acid pH optima, obligate requirement of divalent cations, metalloprotein and glycoprotein nature. Acid optimum pH coupled with the requirement of metal ions prevents their use in chelating buffers or in presence of metal chelators. Hence enzymes which does not show an obligate requirement of divalent cations and has an optimum pH around neutrality would have wider applicability.

The extracellular nuclease from *Basidiobolus haptosporus* (nuclease Bh1) is a high stringency single-strand-specific nuclease which exhibits an optimum pH around neutrality and does not show an obligate requirement of metal ions for its activity and stability. This review gives a detailed account of the characteristics of nuclease Bh1 and its potential as an analytical tool in molecular biology research.

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Enzyme production, purification and assay

Basidiobolus haptosporus (89-3-24) was routinely maintained on MGYP slants. The enzyme production was carried out by inoculating a small piece of the mycelium, from a 24 h old MGYP slant, into 100 ml liquid YPG medium (0.1% w/v yeast extract, 0.5% w/v peptone and 2% w/v glucose), in 500 ml conical flasks, followed by incubation at 30°C on a rotary shaker (200 rpm) for 96 h. The extracellular broth was collected by filtration and used as the source of enzyme (Desai and Shankar, 2000).

The DNase and RNase activities of nuclease Bh1 were determined by measuring the acid soluble nucleotides liberated, at 260 nm, following the hydrolysis of DNA and RNA at pH 8.5 and 37°C. A unit of the enzyme is defined

Table 1. Purification of nuclease Bh1.

Step	Total activity (U)	Total protein (mg)	Sp. activity (U/mg)	Fold purification*	Recovery (%)	ssDNAse : RNase
Crude (ssDNase)	5,00,000	5000.00	100	1.00	100.00	1.00
(RNase)	(5,00,000)					
Ammonium sulfate	4,10,640	217.60	1887	19.13	83.29	37.02
Precipitation and heat Treatment	(11,090)		50			
DEAE-cellulose	2,78,800	9.29	30,000	304.36	56.55	40.12
(pH 7.0)	(6948)		748			
Phenyl-Sepharose	2,00,736	0.81	2,48,128	2516.51	40.71	40.14
(pH 7.0)	(5,000)		5,530			
FPLC on Phenyl	1,28,400	0.22	5,97,209	6056.88	26.04	40.15
Superose (pH 7.0)	(3,198)		14,536			

*Based on ssDNAs activity.
Values in brackets indicate RNase activity.
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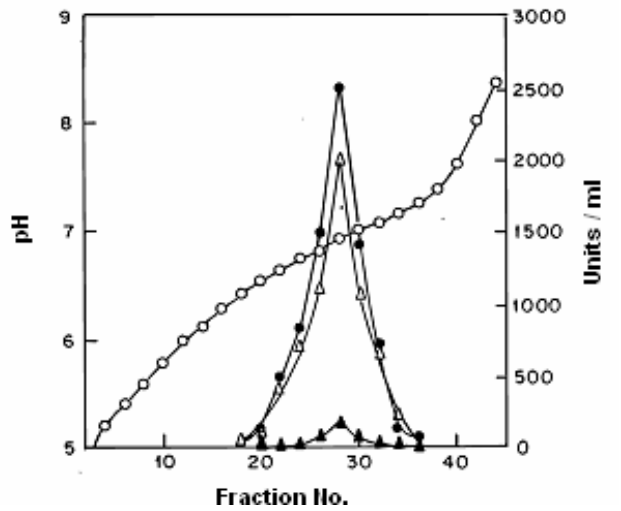


Figure 1. Isoelectric focusing of nuclease Bh1. ssDNase (•), 3'AMPase (Δ) and RNase (▲), pH (o). (Reprinted from Desai and Shankar (2001a), © 2001, with permission form Taylor and Francis.http://www.tandf.co.uk).

on the basis of µmol of acid soluble nucleotides liberated (Desai and Shankar, 2000).

The phosphomonoesterase activity of the enzyme was assayed by measuring the amount of inorganic phosphate released following the hydrolysis of 3'AMP, at pH 8.5 and 37˚C. Unit of the enzyme is defined on the basis of µmol of inorganic phosphate liberated (Desai and Shankar, 2000).

The enzyme was purified to homogeneity with an overall yield of 26% and a specific activity of approximately 600,000 U/mg (Table 1). The decrease in the DNase: RNase ratio, following heat treatment of the ammonium sulfate precipitated and dialyzed enzyme suggested that the major RNase activity is not associated with the DNase activity. Moreover, the constant DNase: RNase ratio in the subsequent steps showed that both the activities are associated with the same protein (Desai and Shankar, 2000).

General properties

Nuclease Bh1 is a multifunctional enzyme with a substrate specificity in the order of ssDNA ≈ 3'AMP >> RNA > dsDNA. All activities of the enzyme showed a pI of 6.8 (Figure 1) suggesting that they are associated with the same protein (Desai and Shankar, 2001a). It is a glycoprotein and contains 15% neutral sugar. The (relative molecular mass) Mr. of the enzyme, determined by gel filtration, was 41 kDa but on SDS/PAGE it gave two bands of Mr 37 kDa and 32 kDa. Glycoprotein staining, after SDS-PAGE, showed them to be glycosylated which on deglycosylation merged into a single band of 30 kDa. Moreover, partial N-terminal sequences of both the bands were identical indicating that the two bands seen on denaturing gel are due to differential glycosylation (Desai and Shankar, 2000). Similar observations were made in case of *Trichoderma viridae* RNase (Inada et. al., 1991) and nuclease PA3 from *Penecillium* sp. (Kazama et al., 1990). Relative molecular mass (Mr.) based on amino acid composition was approximately 32.3 kDa, comparable to that obtained on SDS-PAGE (Desai, 2000). Nuclease Bh1 showed the same pH (8.5) and temperature (60°C) optima for the hydrolysis of all substrates and stability (Desai and Shankar, 2000; Desai and Shankar, 2001a; Desai and Shankar, 2001b). Unlike majority of analytically important single-strand specific nucleases (Desai and Shankar,

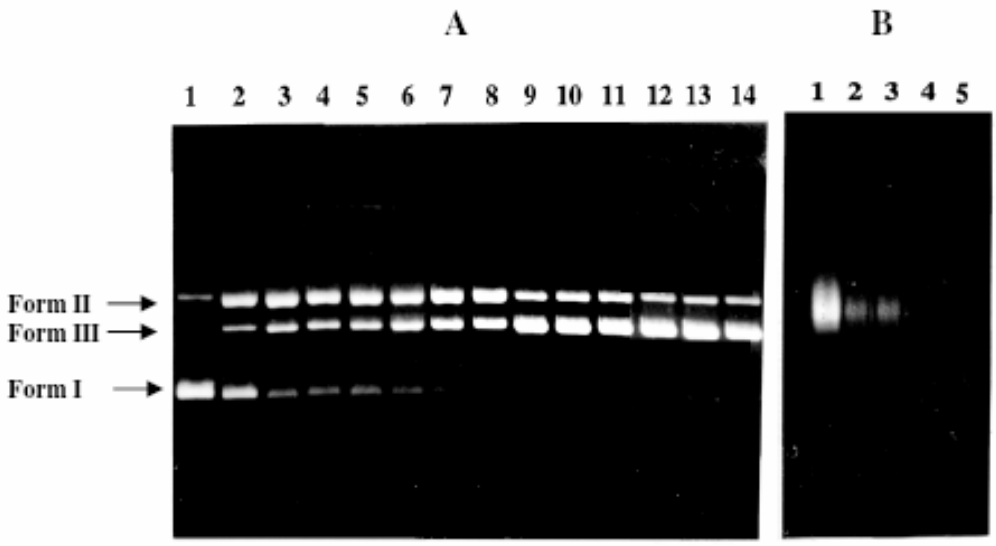


Figure 2. Action of nuclease Bh1 on plasmid pUC 18 and phage M13 DNAs. (A) Time course of digestion of pUC 18 DNA by nuclease Bh1.Lane 1: pUC 18 alone; lane 2: digestion with 0.1 U of nuclease Bh1 for 2 min; lanes 3 - 5: digestion with 0.15 U of nuclease Bh1 for 5, 10 and 15 min. respectively; lanes 6 - 8: digestion with 0.2 U of nuclease Bh1 for 5, 10 and 15 min respectively; lanes 9 - 11: digestion with 0.4 U of nuclease Bh1 for 5, 10 and 15 min respectively; lanes 12 - 14: digestion with 1 U of nuclease Bh1 for 5, 10 and 30 min respectively. (B) Time course of covalently closed single stranded circular phage M13 DNA digestion by nuclease Bh1. Lane 1: phage M13 DNA alone; lanes 2 - 5: digestion of M13 phage DNA with 0.01 U of nuclease Bh1 for 2, 5, 10 and 15 min respectively. (Reprinted from Desai and Shankar (2000), © 2000, with permission form Blackwell Publishing).

2003), nuclease Bh1 did not show an obligate requirement of divalent metal ions for its activity nor its activity stimulated in presence of metal ions. However, like S1 and P1 nuclease (Desai and Shankar, 2003), nuclease Bh1 is a zinc metalloprotein and contains 4 g atoms of zinc/ mol of the enzyme (Desai, 2000). The ssDNase, 3'nucleotidase and RNase activities of nuclease Bh1 were inhibited by metal ions like, $Zn^{2+,}$ $Ag^{2+,}$ Hg^{2+}, Al^{3+} and Fe^{3+} whereas, Mg^{2+}, Mn^{2+},Co^{2+},Ca^{2+} and Ba^{2+} did not have any effect. The enzyme was also inhibited by inorganic phosphate, pyrophosphate, thiol reagents, metal chelators, salts and competitively by guanosine 5'nucleotides. It is stable to high concentrations of organic solvents and urea but susceptible to low concentrations of SDS and guanidine hydrochloride (Desai and Shankar, 2000; Desai and Shankar, 2001a; Desai and Shankar, 2001b).

Specificity and mode of action

DNase activity

Nuclease Bh1 hydrolyzed DNA yielding 5' mononucleotides and oligonucleotides with 3'hydroxyl and 5'phosphoryl termini. Low concentration of the enzyme converted supercoiled pUC18 DNA (form I) to linear duplex DNA (form III) via nicked circular DNA (form II) and linearized covalently closed M13 DNA (Figures 2 A, B) indicating an endo mode of action. Though there was no detectable hydrolysis of linear duplex DNA, even on prolonged incubation, slight degradation could be seen only in presence of 20-fold excess enzyme, pointing towards the high specificity of nuclease Bh1 for single- stranded nucleic acids. Conversion of form I DNA to form III DNA was due to single base nicks as the latter could be ligated with T4 DNA ligase to yield form II DNA. This also suggested that the conversion of form I DNA to form III DNA, by nuclease Bh1, follows a double hit mechanism (Desai and Shankar, 2000). A similar observation was made in case of S1 nuclease (Shishido and Ando, 1981). On the contrary, the nicks generated by BAL 31 nuclease on pBR322 form I DNA could not be ligated as they were extended to gaps by the exonuclease action of the enzyme (Przykorska et al., 1988). This observation coupled with the ability of T4 DNA ligase to convert pUC 18 form III DNA to form II DNA, obtained following the action of nuclease Bh1 (Desai and Shankar, 2000), can also be correlated to the absence of exonucleolytic activity in nuclease Bh1.

Time course analysis of the hydrolytic products of ssDNA, with low enzyme concentration, showed the predominance of 5'dGMP followed by 5'dTMP whereas, in presence of high concentration of the enzyme, small amounts of 5'dAMP could also be seen. In both the cases, after an initial surge, the reaction slowed down considerably and there was no detectable increase in the

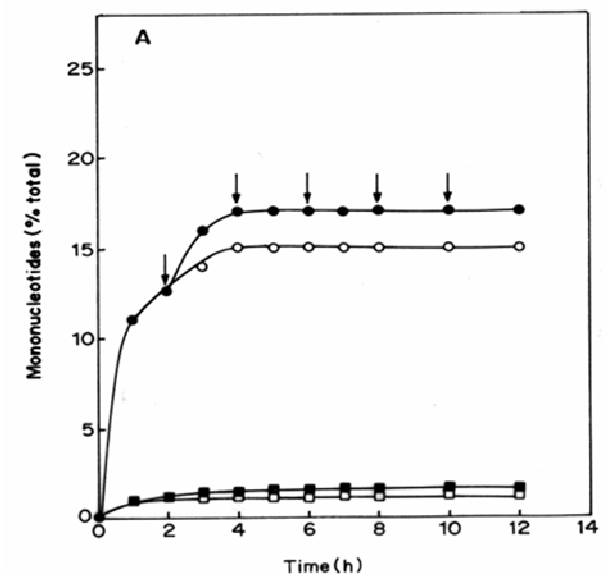


Figure 3A. Time course of hydrolysis of ssDNA with low concentrations (6 U/ml) of nuclease Bh1. Without dosage - 5'dGMP (●), 5'dTMP (□). With dosage - 5'dGMP (●), 5'dTMP (■). The position of the arrow indicates the addition of the nuclease Bh1 (6 U/ml). (Reprinted from Desai and Shankar (2000), © 2000, with permission form Blackwell Publishing).

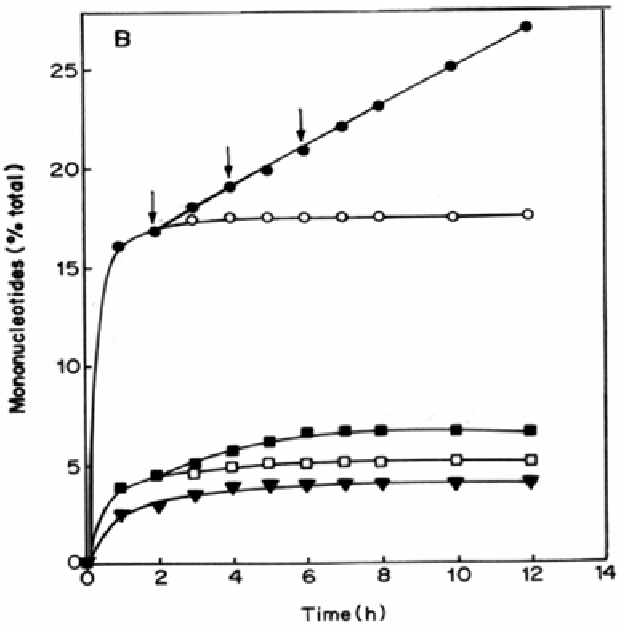


Figure 3B. Time course of hydrolysis of ssDNA with high concentrations (60 U/ml) of nuclease Bh1. Without dosage - 5'dGMP (O), 5'dTMP (□), 5'dAMP (▼). With dosage - 5'dGMP (●), 5'dTMP (■), 5'dAMP (▼). The position of arrow indicates the addition of nuclease Bh1 (60U/ml). (Reprinted from Desai and Shankar (2000), © 2000, with permission form Blackwell Publishing).

mononucleotide levels (Figure 3 A, B). This suggested product inhibition rather than depletion of the substrate as 5'dGMP is a competitive inhibitor of nuclease Bh1. Moreover, with high enzyme concentration, periodic dosing of the enzyme (i.e. incremental addition the enzyme at fixed time intervals) resulted in a marked increase in the 5'dGMP levels along with a slight increase in the 5'dTMP (Figure 3B), whereas this was not observed with low enzyme concentration (Figure 3A), thus pointing towards the inability of the latter to overcome 5'dGMP inhibition (Desai and Shankar, 2000).

Analysis of the hydrolytic products of dsDNA, with low enzyme concentration, showed the presence of nucleotides in the order of 5'dGMP>5'dTMP>5'dAMP. Similar observations were made when the hydrolysis was carried out in presence of high enzyme concentration except 5'dTMP levels almost equaled that of 5'dGMP (Figure 4 A, B).

During the entire duration of the hydrolysis of ssDNA with high concentration of nuclease Bh1, with and without dosage, the proportion of oligonucleotides was higher than that of mononucleotides. In addition, time course analysis of (dT_{10}) revealed that 5'd TMP could be detected only after 2 h. These observations coupled with the hydrolytic pattern of pUC18 suggested an endo mode of action (Desai and Shankar, 2000). Nuclease α from *Ustilago maydis* (Holloman et al., 1981) and BAL 31 nuclease (Gray et al., 1981) hydrolyzed ssDNA endonucleolytically but showed an exo mode of action on dsDNA whereas, nuclease Bh1 cleaved ss- and dsDNA endonucleolytically (Desai and Shankar, 2000).

Analysis of the hydrolytic products of DNA, with low and high enzyme concentrations of nuclease Bh1, showed high levels of 5'dGMP, from the initial stages of hydrolysis, indicating the preference of the enzyme for guanylic acid linkages. Low levels of 5'TMP and 5'AMP can be due to the relative resistance of thymidylic and adenylic acid linkages to cleavage. Irrespective of the enzyme concentration, the hydrolytic products of DNA showed the absence of 5'dCMP. In addition, analysis of the alcohol soluble and alcohol precipitable oligonucleotides, obtained after exhaustive digestion of DNA, on treatment with snake venom phosphodiesterase, showed high levels of 5'dCMP, showing the resistance of cytidylic acid linkages to cleavage (Desai and Shankar, 2000).

Action of nuclease Bh1 on DNA and deoxyribodecamers revealed that they were hydrolyzed in the order of ssDNA > $(dT)_{10}$ > $(dG)_{10}$. Detectable hydrolysis of $(dA)_{10}$ was observed in presence of 5-fold excess enzyme while $(dC)_{10}$ was resistant to hydrolysis even in presence of 10-fold excess enzyme. ssDNA was hydrolyzed 1.6-1.7 fold faster than deoxydecamers, while 4-fold excess enzyme was required to effect 25 -100% hydrolysis of dinucleoside monophosphates thus pointing towards the high preference of nuclease Bh1 for high molecular weight substrates (Desai and Shankar, 2000).

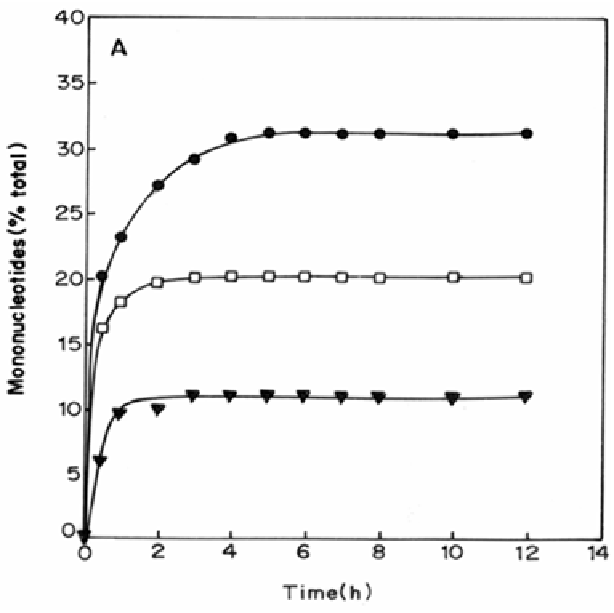


Figure 4A. Time course of hydrolysis of dsDNA with low concentrations (6U/ml of dsDNase activity) of nuclease Bh1. 5'dGMP (●), 5d'TMP (■), 5'dAMP (▼). (Reprinted from Desai and Shankar (2000), © 2000, with permission form Blackwell Publishing).

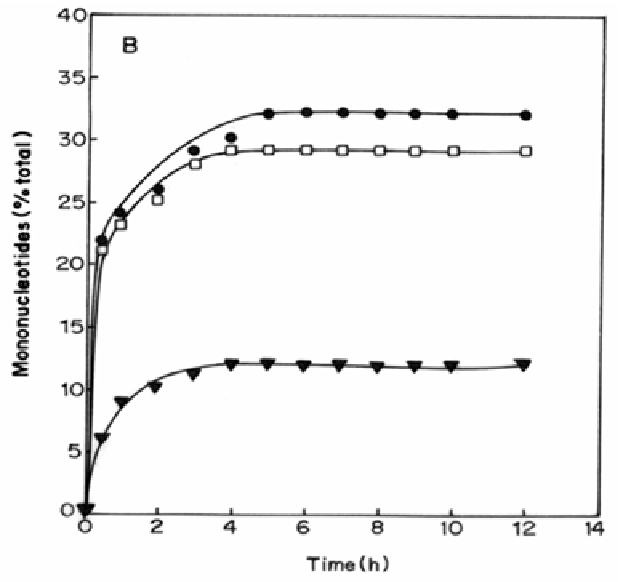


Figure 4B. Time course of hydrolysis of dsDNA with high concentrations (60U/ml of dsDNase activity) of nuclease Bh1. 5'dGMP (●), 5d'TMP (□), 5'dAMP (▼). (Reprinted from Desai and Shankar (2000), © 2000, with permission form Blackwell Publishing).

Time course analysis of DNA by nuclease Bh1 revealed the presence of nucleotides in the order of 5'GMP > 5'TMP > 5'AMP with the absence of 5'CMP. Moreover, the action of nuclease Bh1 on dinucleoside monophosphates showed that homodimers were hydroly-zed in the order of d(GpG) > d(TpT) > d(ApA). Although, d(GpG) was completely hydrolyzed d(CpC) was resistant to cleavage (Figure 5). The type of base present at the 5'end influenced the cleavage efficiency because dinucleoside monophosphates with guanosine at the 5'end were hydrolyzed more efficiently than the ones with thymidine or deoxyadenosine at the 5'end that is, d(GpT) > d(TpG) > d(ApG). Furthermore, the enzyme showed higher preference for dinucleoside monophosphates with deoxyguanosine at the 5' end than when it was present at the 3' end i.e. d(GpT)>d(TpG)>d(ApG). Dinucleoside monophosphates namely, d(TpT) , d(TpA) and d(GpA) were cleaved to a similar extend while d(TpG) and d(GpT) were hydrolyzed to a greater extend showing that internucleotide bonds between dT and dG are more susceptible than those between dT and dA or dG and dA. In contrast, dinucleoside monophosphates with cytidine either at the 3' or 5' end were resistant to hydrolysis (Figure 5).

3' / 5'	dG	dT	dA	dC
dG	100	79	52	0
dT	65	53	54	0
dA	35	40	25	0
dC	0	0	0	0

Figure 5. Hydrolysis of deoxyribodinucleoside mono-phosphates by nuclease Bh1. The numbers indicate per-cent hydrolysis. (Reprinted from Desai and Shankar (2000), © 2000, with permission form Blackwell Publis-hing).

Action of nuclease Bh1 on dinucleoside monophosphates and ssDNA showed high preference for guanylic acid linkages. This observation coupled with the complete hydrolysis of d(GpG) accompanied by a surge in the 5'dGMP levels, on periodic dosing , as well as the preference of the enzyme for dinucleoside monophos-phates in the order of d(GpG)>d(GpT)>(TpG) indicated the preference of nuclease Bh1 for G stretches in ssDNA. On the contrary, *Streptomyces antibioticus* nuclease preferred G stretches in dsDNA and not in ssDNA. Moreover, as observed with nuclease Bh1, analysis of the hydrolytic products of dsDNA by *S. antibiotics* nuclease (Cal et al., 1996) and a single-strand-specific nuclease from *Chymadomonas* sp. (Burton et al., 1977) showed the preponderance of G followed by T indicating that after d(GpG) linkages, d(GpT) linkages are preferred. The resistance

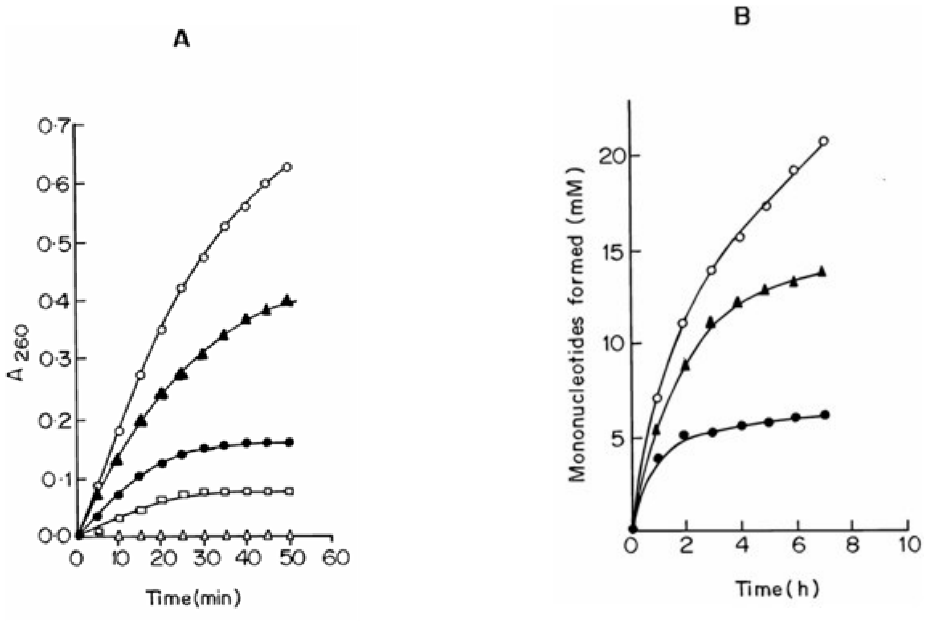


Figure 6. Time course of hydrolysis of homoribopolynucleotides and RNA by nuclease Bh1. (A) Hydrolysis of polynucleotides and RNA. Poly A (o), poly U (▲), RNA (•), poly[A].poly[U] (□), poly G and ploy C (Δ). (B) Release of 5' mononucleotides from RNA. 5'AMP (o), 5'UMP (▲), 5'GMP (•). (Reprinted from Desai and Shankar (2001b), © 2001, with permission from Taylor and Francis. http://www.tandf.co.uk).

of $d(C)_{10}$ and dinucleoside monophosphates with cytosine either at the 3, or 5' termini to cleavage coupled with high amounts of amounts of 5'dCMP in the in the oligonucleotides remaining after exhaustive digestion of DNA, by nuclease Bh1, explains the resistance of cytidylic acid linkages to hydrolysis (Desai and Shankar, 2000).

RNase activity

Nuclease Bh1 hydrolysed homoribopolynucleotides and RNA in the order of poly A > poly U > RNA > poly A poly U whereas, poly C and poly G were resistant to cleavage. The low rate of hydrolysis of poly A; poly U (approx. 16% that of poly A) suggested the preference of the enzyme for single-stranded nucleic acids (Desai and Shankar, 2001b).

Hydrolytic products of RNA, by nuclease Bh1, yielded 5' mononucleotides and oligonucleotides with 3'hydroxyl and 5'phosphoryl termini. Time course analysis of the hydrolytic products of RNA revealed the presence of 5'mononucleotides in the order of 5'AMP > 5'UMP > 5'GMP (Figure 6). This observation along with the high susceptibility of poly A suggested the high preference of the enzyme for adenylic acid linkages. The ssDNase and RNase activities of *N. crassa* nuclease (Linn and Lehman, 1965) showed preference for guanylic acid linkages while wheat chloroplast nuclease (Kuligowska et al., 1988) preferred adenylic acid linkages. However, nuclease Bh1 exhibited different base preference with ssDNA and RNA (Desai and Shankar, 2000; Desai and Shankar, 2001b). As observed in case of DNA hydrolysis, 5'CMP could not be detected in the hydrolytic products of RNA. Moreover, the oligonucleotides obtained after exhaustive digestion of RNA, on treatment with snake venom phosphodiesterase, showed the predominance of 5'CMP. These observations coupled with the resistance of poly C indicated the resistance of cytidylic acid linkages to cleavage (Desai and Shankar, 2001b).

Analysis of the hydrolytic products of RNA revealed high proportion of oligonucleotides than mononucleotides during the initial phase of hydrolysis. However, as the hydrolysis progressed, the proportion of mononucleotides increased pointing towards an endo-exo mode of action (Desai and Shankar, 2001b). Wheat seedlings nuclease hydrolyzed ssDNA endonucleolytically but showed an exo mode of action on RNA (Kroeker et al., 1975) whereas wheat chloroplast nuclease (Kuligowska et al., 1988) cleaved both ssDNA and RNA endonucleolytically. In contrast, nuclease Bh1 exhibited an endo mode of action on DNA but an endo-exo mode of action on RNA (Desai and Shankar, 2000; Desai and Shankar, 2001b).

Nucleotidase activity

Action of nuclease Bh1 on various 2', 3' and 5' ribo- and deoxyribomononucleotides as well as 2', 3'- and 3', 5' cyclicnucleotides, showed it to be 3' ribonucleotide specific. Moreover 3' mononucleotides were dephosphorylated in the order of 3' UMP > 3' AMP >> 3'GMP >> 3' CMP (Desai and Shankar, 2001a). S1 and mungbean nuclease

Table 2. Kinetic parameters for the hydrolysis of 3'-nucleotides by nuclease Bh1.

Substrate	K_m (µmoles)	V_{max} (µmoles.min^{-1}.mg^{-1}) x 10^5	V_{max}/K_m	K_{cat}x 10^{10}
3'AMP	92.00	5.71	0.062	6.54
3'UMP	100.00	8.40	0.082	9.62
3'GMP	125.00	3.30	0.026	3.78
3'CMP	6250.00	1.67	0.0002	1.91
pAp	95.00	5.80	0.061	6.53

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(Oleson and Hoganson, 1981; Kole et al., 1974) hydrolyzed 3'-ribinucleotides faster than deoxyribonucleotides and this was correlated to the presence of 2'-OH group. In contrast, with nuclease Bh1, deoxyribonucleotides were resistant to cleavage as observed with 3'nucleotidase/nuclease from *Crithidia luciliae* (Neubert and Gottlieb, 1990). Moreover 3' mononucleotides were dephosphorylated in the order of 3' UMP > 3' AMP >> 3'GMP >> 3' CMP (Desai and Shankar, 2001a). In case of S1 and mungbean nuclease (Oleson and Hoganson, 1981; Kole et al., 1974) presence of a 5'phosphomonoester group or a substituent in 3'AMP increased the rate of hydrolysis while 3'nucleotidase-nuclease from *C. luciliae* (Neubert and Gottlieb, 1990) hydrolyzed pAp approximately half the rate of 3'AMP. On the contrary, 5' subsistent in 3'AMP did not influence the cleavage efficiency of nuclease Bh1 since both 3'AMP and pAp cleaved at a similar rate (Table 2).

Although the affinity for 3'AMP was marginally higher than that of 3' UMP, the V_{max} for 3'UMP was 1.5-fold higher than that of 3'AMP indicating the higher rate of hydrolysis of 3' UMP. The low rate of hydrolysis of 3'GMP can be due to its acting as an inhibitor analog since 5'GMP is a competitive inhibitor of nuclease Bh1. The high K_m and K_{cat} for 3'CMP indicates its resistance to cleavage (Table 2).

Active Site

Nuclease Bh1 catalyzes the hydrolysis of ssDNA, 3'AMP, and RNA in a ratio of approximately 1:1: 0.05. The phosphomonoesterase activity of the enzyme was inhibited competitively by ssDNA and RNA suggesting the presence of a common catalytic site for the hydrolysis of both monomeric and polymeric substrates (Desai and Shankar, 2001a). Moreover, chemical modification studies showed the involvement of a single lysine and carboxylate residue in the catalytic activity of the enzyme. The substrates of nuclease Bh1 viz. ssDNA, 3'AMP and RNA and 5'GMP (the competitive inhibitor) could protect the enzyme against trinitrobenzenesulfonic acid (TNBS)-mediated inactivation whereas, substrate and inhibitor protection was not observed in case of carbodiimide (EDC)-mediated inactivation of the enzyme. Moreover, the lysine modified enzyme, having very little catalytic activity, showed a significant decrease in its ability to bind 5'GMP, while the carboxylate modified enzyme (having very little catalytic activity) could effectively bind 5'GMP, pointing towards the involvement of lysine in substrate binding and carboxylate in catalysis. The parallel loss of all three activities of nuclease Bh1, on lysine and carboxylate modification, coupled with substrate and competitive inhibitor protection and competitive inhibitor bind-ing data confirmed the presence of a common catalytic site for the hydrolysis of both monomeric and polymeric substrates (Desai and Shankar, 2001c). Similar observations were reported for S1 nuclease (Gite et al., 1992a, b).

Conclusion

Nuclease Bh1 is a high stringency single-strand-specific nuclease which exhibits high preference for guanylic acid linkages in DNA and does not show an obligate requirement of divalent cations for its activity and stability. Its high stability to pH, temperature, high concentrations of urea and organic solvents suggests that it can be used in a wide variety of experimental conditions for the analysis of DNA structure. Since the enzyme activity is not affected by divalent cations viz. $Mg^{2+,}$ $Mn^{2+,}$ Ca^{2+} and Co^{2+}, it can be used in conjunction with metal requiring enzymes like restriction endonucleases and topoisomerases (Desai and Shankar, 2000). Moreover, the resistance of cytidylic acid linkages to cleavage can be exploited for the isolation of C-streches in DNA.

In general, the properties of nuclease Bh1 viz. metalloprotein and glycoprotein nature, high pH and temperature stability, multifunctional activity, high single strand specificity, mode of action and end product inhibition are comparable to single-stranded-specific enzymes like S1, P1, *N. crassa* and barley nucleases. In addition, partial N-terminal sequence of nuclease Bh1 showed considerable similarity with single-strand-specific nucleases viz. S1, P1, PA 3, Le1, Le3 and barley nuclease (Desai and Shankar, 2000). Hence, nuclease Bh1 can be assigned to the class EC 3.1.30.1.

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