Vol. 121, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 31, 1984 Pages 62-68

THE DNASE ACTIVITY OF AN ENDOPLASMIC RETICULUM NUCLEASE AND ITS EFFECT ON DNA SYNTHESIS IN VITRO

Athanasios Triantos, Sofia Kouidou, George Papageorgiou, Evangelos Kavoukopoulos and Antonios Trakatellis

Department of Biochemistry, School of Medicine, Aristotelian University of Thessaloniki, Thessaloniki, Greece

Received March 20, 1984

SUMMARY: An endoplasmic reticulum nuclease which was isolated previously in this laboratory from rat liver (Kouidou et al. (1981) Eur.J.Bioch. 120, 9-14) was found to degrade linear and circular single stranded DNA but not double stranded DNA. The DNA fragments resulting from this cleavage were longer than 20 nucleotides. In addition the nuclease was found to improve the efficiency of DNA template used by DNA polymerase I in DNA synthesis in vitro. The results were the same whether incubation of the template with the nuclease was prior to addition of DNA polymerase I or simultaneously with polymerization. When nuclease was added after the completion of polymerization by DNA polymerase I it was ineffective unless the product was denatured. These data further corroborate the observation that double stranded DNA is not cleaved by this enzyme.

Deoxyribonucleases (DNases) have been shown to play a significant role in nucleic acid metabolism including replication, repair, recombination and restriction (1-5). Most of these enzymes have been isolated from procaryotic sources but less in known about eucaryotic DNases (6-9). Furthermore,whole cells or isolated nuclei have been used as a source for the eucaryotic enzymes which are referred to above, and there is relatively little information about similar DNases of different cellular localization (5,10,11). It was reported in a previous paper from this laboratory that an endoplasmic reticulum nuclease purified from rat liver (12), possesses RNase and DNase activity. The DNase activity of this enzyme is further investigated in this report and its role on DNA synthesis in vitro is studied.

0006-291X/84 $1.50

MATERIALS AND METHODS

Materials: N,N-methylene bisacrylamide, and N,N,N',N'-tetramethylethylenediamine all high purity, were purchased from Ames Company. Acrylamide, and ammonium persulfate also of high purity were purchased from Serva.

Radioactive labeled nucleotides (^{3}H)dCTP (21CI/mmol) and (^{3}H)UTP (17Ci/mmol) and poly (^{3}H)uridylic acid (20-72 Ci/mmol) were purchased from Amersham Radiochemicals.

Enzymes: DNA polymerase I (specific activity 2500-5000 units/mg) was purchased from Boeringer Mannheim. Endoplasmic reticulum nuclease was prepared according to Kouidou et al. (12).

DNA substrates: Plasmid PBR 322 from E.coli was purchased from Boeringer Mannheim; calf thymus DNA was a generous gift of Dr. O.Antonoglou. Radioactively labeled DNA was synthesized by DNA polymerase I according to Loeb (13), using calf thymus DNA as a template and (^{3}H)dCTP. The high molecular weight material was isolated on a G-50 collumn and subsequently purified by phenol-chloroform extraction.

Buffers: Buffer A (Tris/glycerol/EDTA/dithiothreitol) contained 50mM Tris/HCl (ph 8.3), 0.25mM EDTA, 0.5mM dithiothreitol and 20% glycerol. Buffer LS contained 10mM Tris/HCl (pH 7.5), 10mM $MgCl_2$, and 1mM dithiothreitol.

Enzyme Assays: DNA polymerase I assay mixture was prepared according to Loeb (13). Degradation of radioactively labeled DNA (0,5μg) was tested in LS buffer containing 0.05% bovine serum albumin and 10μg of enzyme preparation (total volume 40μl). The mixture was incubated at 37°C for 1h. 35μl aliquots were then taken from this mixture, loaded on paper discs(14) and measured for TCA insoluble radioactivity according to Bollum (15), unless otherwise noted.

DNA electrophoresis was done in 4% polyacrylamide gels according to Maniatis et al.(16) with minor modifications. Prior to electrophoresis 5μg DNA were incubated in LS buffer containing 0.05% BSA and 50μg endoplasmic reticulum nuclease preparation (total volume 100μl), at 37°C for 1h. The samples were subsequently purified according to Maniatis et al.(17) and subjected to electrophoresis.

Denatured DNA was prepared by boiling native DNA for 10min and then immediately chilling the sample on ice.

RESULTS AND DISCUSSION

The effect of endoplasmic reticulum nuclease on different kinds of denatured DNA is shown in Fig. 1. It is evident from this figure that the enzyme can degrade denatured DNA such as calf thymus DNA and plasmid PBR 322 DNA which does not possess single stranded ends. Indeed in columns I and 2 (Fig.1) which were obtained with calf thymus DNA of a rather broad range of molecular weights one can observe that after treatment with nuclease the high molecular weight region disappears and the smaller DNA fragments migrate further into the gel. In column

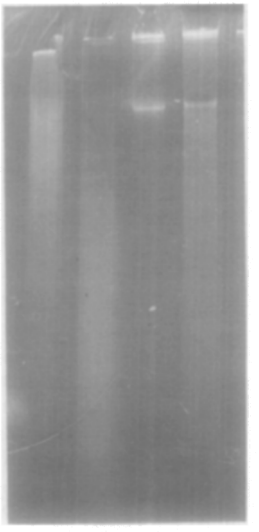

Figure 1. Polyacrylamide gel electrophoresis of denatured DNA from different sources before and after degradation by endoplasmic reticulum nuclease. Positions (1) and (3) contained calf thymus and plasmid PBR 322 respectively; the samples were incubated in the absence of endoplasmic reticulum nuclease prior to gel electrophoresis. Positions (2) and (4) contained DNA from the same sources as positions (1) and (3) respectively, but the samples were digested with endoplasmic reticulum nuclease before subjected to gel electrophoresis. For details see Materials and Methods.

3 a single band is observed which represents denatured plasmid PBR 322 DNA prior to digestion. Column 4 shows the electrophoretic behavior of denatured PBR 322 DNA after digestion with the nuclease. One can observe that fragments of lower molecular weight which migrate further into the gel are now visible. In addition part of the PBR 322 DNA remains undigested but the intensity of the main band is reduced. Since even prolonged digestion did not lead to complete disappearance of the main band one may say that this band represents fully renatured PBR 322 DNA which is resistant to degradation by the endoplasmic reticulum nuclease. In contrast native DNA from the same sources was found to be insensitive to the nucleolytic activity of the same enzyme (results not shown). These results show that the endoplasmic reticulum nuclease which has been

TABLE I

DENATURED DNA* DEGRADATION BY ENDOPLASMIC RETICULUM NUCLEASE

	(-)Endoplasmic reticulum nuclease	(+)Endoplasmic reticulum nuclease
Precipitable radioactivity in 5% TCA**(cpm)	7.600[a]	3.900
Precipitable radioactivity in 20% TCA***(cpm)	7.500[a]	7.380

* Denatured calf thymus DNA was used as a substrate for these experiments. Other conditions are outlined in the text.
** TCA insoluble product measured according to Bollum(15).
*** TCA insoluble product measured according to Maniatis et al.(18).
[a]This radioactivity represents more than 95% of DNA radioactivity in solution.

isolated in this laboratory possesses endonucleolytic activity towards single stranded DNA.

The endonucleolytic nature of the enzyme was reinforced by the following observations. When (^{3}H)DNA is treated with endoplasmic reticulum nuclease, no acid soluble radioactivity could be detected when 20% TCA was used for precipitating the DNA product (18) (Table I); if however, 5% TCA containing 1% $Na_4P_2O_7.10H_2O$ (15) was used for precipitation, approximately 50-60% of the radioactivity was found to be acid soluble. In view of the fact that DNA fragments that contain less than twenty nucleotides are soluble in 20% TCA (18) it can be concluded that the enzyme under study does not produce such fragments and therefore does not exhibit any significant exonucleolytic activity.

Pretreatment of a DNA template with a nuclease that degrades single stranded DNA producing 3'OH ends (bovine pancreas DNase I) is known to increase the synthesis of DNA by DNA polymerase I (19). Similarly, when DNA was incubated with the purified nuclease from endoplasmic reticulum and the nucleolytic enzyme was inactivated by heating at 60°C after digestion,

TABLE II

EFFECT OF DNA PREINCUBATION WITH ENDOPLASMIC RETICULUM NUCLEASE ON THE SYNTHESIS OF DNA BY DNA POLYMERASE I.

	Incorporation of (^{3}H)dCTP by DNA polymerase I		
	DNA not treated with nuclease[a]	Nuclease added during polymerization[b]	DNA preincubated with nuclease[c]
DNA polymerase I product(counts/min)	36.500	62.530	63.680

[a]Sheared and denatured calf thymus DNA(10µg) was incubated for 20min at 37°C with Tris-Maleate buffer (pH 8.0) 8.33µmol, containing KCl 0.1µmol, $MgCl_2$ 1µmol, ß-mercaptoethanol 50nmol. The mixture was then heated at 100°C for 10 min and chilled on ice for 5min. To this mixture dATP,dCTP, dGTP and dTTP 0,33nmol each, (^{3}H)dCTP 1µCi, E.coli DNA polymerase I (1 unit) and 20µl buffer A were added (final volume 55µl). The mixture was then incubated for 30min at 37°C and the TCA insoluble radioactivity was estimated.

[b]Same as a but endoplasmic reticulum nuclease (0.8µg) was substituted for buffer A.

[c]Same as b but endoplasmic reticulum nuclease was added to the incubation mixture together with the DNA before the second incubation step.

incorporation of (^{3}H)dCTP to the DNA synthesized by DNA polymerase I was 100% higher than that observed with untreated DNA template (Table II). This effect was maximal after 60 min of preincubation; further DNA preincubation up to 3h did not affect the incorporation of (^{3}H)dCTP (results not shown). These findings strongly indicate that the enzyme under study produces relatively large DNA fragments which possess 3'OH ends and which are appropriate primers for DNA synthesis by DNA polymerase I and rat liver low molecular weight DNA polymerase (results not shown).

Endoplasmic reticulum nuclease was also found to increase DNA synthesis in vitro when added during the course of polymerization together with DNA polymerase I in the reaction mixture (Fig.2). The same effect was observed when low molecular weight DNA polymerase from rat liver was used instead of DNA polymerase I (results not shown). Under these conditions incorporation of (^{3}H)dCTP into DNA was found to increase more than 100%

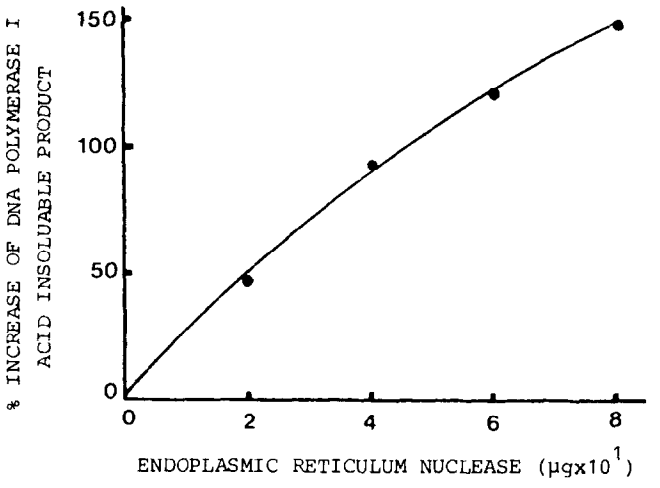

Figure 2. Increase of (^{3}H)dCTP incorporation in DNA synthesized by DNA polymerase I in the presence of different concentrations of endoplasmic reticulum nuclease. The assay mixture contained Tris-Maleate (pH 9.0) 8.33mmol; KCl 0.1mmol; $MgCl_2$ 1mmol; β-mercaptoethanol 50nmol; dATP, dCTP, dGTP and dTTP 0.33nmol each; sheared and denatured calf thymus DNA 10μg; (^{3}H)dCTP (1μCi) and DNA polymerase I (1 unit). To this mixture 20μl buffer A (control) or different concentrations of nuclease in buffer A were added (total volume 55μl). Percent increase of (^{3}H) dCTP incorporation expresses the ratio counts/min obtained in the presence of nuclease vs counts/min of control.

in the presence of nuclease. This finding suggests that the nuclease under study causes 3' cleavage, in other words produces more free 3' OH ends in DNA primers and also indicates that the degradation of the double stranded replication product must be insignificant.

ACKNOWLEDGEMENTS

The authors are indebted to Ms A.Georgiadou for her expert typing. This work was supported by a grant from the Ministry of Research and Technology of Greece.

REFERENCES

1. Frencke B. and Ray D.S.(1971) J.Mol.Biol. 61, 565-586
2. Waldstein E.A., Peller S. and Setlow R.B.(1979) Proc.Natl. Acad.Sci.USA 76, 3746-3750.
3. Rogers S.G. and Rhoades M.(1976) Proc.Natl.Acad.Sci. USA 73,1576-1580.
4. Angel T., Austin B. and Catcheside,D.G.(1970) Aust.J.Biol. Sci. 23,1229-1240.
5. Rosamond J.(1981) Eur.J.Biochem. 120, 541-546.
6. Mckenna W.G., Maio J.J. and Brown F.L.(1981) J. Biol. Chem. 236, 6435-6443.
7. Hashida T., Tanaka Y., Matsunami N., Yoshihara K., Kamiya T., Tanigawa Y. and Koide S.S. (1982) J. Biol. Chem. 257, 13114-13119.

8. Shaper N.L., Gatsom R.H. and Grossman L.(1982) J. Biol. Chem. 257, 13455-13458.
9. Preykorka J., Szarkowski J. (1980) Eur. J. Biochem. 108, 285-293.
10. Foury F.(1982) Eur. J. Biochem. 124, 253-259.
11. Tigesstrom R.G. (1982) Biochemistry 21, 6397-6403.
12. Kouidou S., Triantos A., Kavoukopoulos E. and Trakatellis A. (1981) Eur. J. Biochem. 120, 9-14.
13. Loeb L.A. (1969) J. Biol. Chem. 244, 1672-1681.
14. Antonoglou O., Salakidou H., Haralambidou E. and Trakatellis A. (1977) Biochim. Biophys. Acta 474, 467-477.
15. Bollum F.J. (1968) Methods Enzymol. 12B, 169-173.
16. Maniatis T., Jeffrey A. and Kleid D.E.(1975)Proc. Natl. Acad. Sci. USA 72, 1184-1188.
17. Maniatis T., Fritsch E.F., Sambrook J., Molecular Cloning. (1982) Cold Spring Harbor Laboratory, p. 474.
18. Maniatis T., Fritsch E.F., Sambrook J., Molecular Cloning. (1982) Cold Spring Harbor Laboratory, p.473.
19. Maniatis T., Fritsch E.F., Sambrook J., Molecular Cloning. (1982) Cold Spring Harbor Laboratory, p.142.