S_1 NUCLEASE CONVERTS RELAXED CIRCULAR CLOSED DUPLEX DNA TO OPEN LINEAR DNA OF UNIT LENGTH

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1. Introduction

Superhelical DNA can be cleaved open by the single-strand-specific S_1 nuclease from Aspergillus oryzae (EC 3.1.4.X) to form linear duplexes of unit length [1–4]. The suggestion was made, that, owing to a topological constraint in these molecules partially single-stranded structures might be revealed as sites of attack for the S_1 nuclease [1–3]. Introduction of one or more single-strand nicks into a superhelical molecule will cause relaxation of the circular DNA and, concordingly, removal of putative single-stranded regions. Such relaxed molecules should no longer be converted to linear duplexes of unit length (form III) by S_1 nuclease if the above-mentioned hypothesis were correct. We have shown, however, that nicked circular SV40 DNA (form II) can, nevertheless, be cleaved open to form III by the enzyme, despite the absence of partially weakly base-paired regions and regardless of the number of single-strand nicks present [5]. To test further the hypothesis that S_1 nuclease requires single-stranded regions in order to introduce a double-strand break into circular DNA, we have employed synthetic circular fd replicative intermediate (RF) DNA [6] as a substrate. This double-stranded DNA is relaxed, is devoid of nicks and of topological constraints, and should, therefore, provide an interesting substrate for the double-strand cleaving activity in S_1 nuclease preparations. We will show, that in disagreement with the current hypothesis, S_1 nuclease does not require single-stranded regions in order to exert its double-strand-cleaving activity on circular DNA, since fd RF DNA is converted to form III by treatment with S_1 nuclease.

2. Materials and methods

Relaxed double-stranded circular fd RF DNA [6] was kindly provided by H. Schaller and C. Gray, Institut für Mikrobiologie, Universität Heidelberg. ^{3}H-Labeled superhelical SV40 DNA form I was isolated and purified as described [7]. Preparation and assay of S_1 nuclease have been described elsewhere [5,4]. The use of S_1 buffer with higher salt (100 mM NaCl instead of 50 mM) had no influence on the results. We have also shown that the sedimentation coefficient of the fd RF DNA and of DNase-treated [5] DNA remained unaltered in S_1 buffer by cosedimenting these DNAs together with superhelical SV40 FO I DNA through neutral 5 to 20% sucrose gradients in S_1 buffer (data not shown here). This demonstrates that the S_1 buffer does not affect the conformation of the fd RF DNA by introducing superhelicity. Electrophoresis in 1.4% agarose gels were as described [5]. Prior to electrophoresis the samples were mixed with one tenth the reaction volume of a solution containing 8 M urea, 0.1 M EDTA and 0.05% Bromophenol Blue in 50% sucrose. Alkaline velocity sedimentation was performed in a 5 to 20% sucrose gradient, pH 12.7, containing 1 M NaCl, 0.1 M Tris, 0.01 M EDTA and 1.5 μg unlabeled calf thymus DNA. Centrifugation took place in a Spinco SW65 rotor at 63 000 rev/min for 70 min.

3. Results and discussion

To ascertain that the fd RF DNA preparation consisted of covalently closed circular molecules that

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did not contain any single-strand nicks which might serve as sites of attack for the S_1 nuclease [8] the following experiment was performed: ethidium bromide (EtBr) was added to an aliquot of the fd RF DNA in order to test if, and, to which extent the DNA preparation could be converted to superhelical structures. Agarose gel electrophoresis revealed that almost all molecules were devoid of nicks, as they could be converted to fast moving supercoiled structures (fig.1, open circles). For control, fd RF was subjected to a limited DNase-treatment, mixed with EtBr and electrophoresed. As expected, introduction of nicks prevented formation of supercoils (fig.1, filled circles).

To test whether S_1 nuclease is capable of cleaving open fd RF to linear duplexes of unit length, ^{32}P-fd RF was mixed with ^{3}H-labeled superhelical SV40 DNA (which is known to be converted by S_1 to form III) and the reaction mixture was electrophoresed after S_1 treatment in agarose (fig.2a). It may be seen that fd RF (filled circles) is, indeed, opened by a double-strand break to linear unit length form III by S_1 nuclease which coelectrophoresis together with a Hind

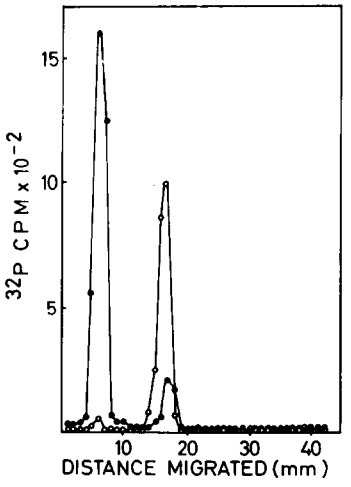

Fig.1. Agarose gel electrophoresis of fd RF DNA in presence of ethidium bromide (0.5 μg/ml) in electrophoresis buffer. Prior to electrophoresis, the samples were adjusted to a final concentration of 0.05 μg/ml in ethidium bromide. (• – •) fd RF DNA + DNase. The reaction mixture (90 μl) contained 70 μl (4500 cpm, 0.16 μg) of ^{32}P fd RF, 10 μl 10 × TEG buffer (100 mM Tris–HCl, pH 7.2, 100 mM $MgCl_2$, 10 mM EDTA and 50% glycerol) and 10 μl DNase (0.05 μg/ml in TEG buffer). After 20 min at 0°C the reaction was terminated by the addition of 10 μl 0.2 M EDTA. (○ –○) without DNase.

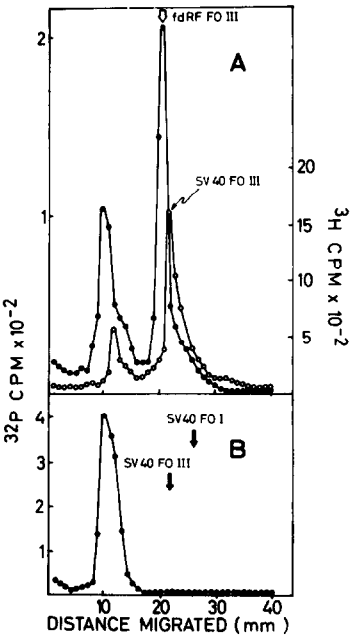

Fig.2. Agarose gel electrophoresis of S_1 treated ^{32}P fd RF DNA and ^{3}H-labeled SV40 FO I DNA. Gels A and B were electrophoresed in parallel tracks within the same gel. (A) The reaction mixture (63 μl) contained 40 μl ^{32}P fd DNA (2500 cpm, 0.09 μg), 7 μl ^{3}H-labeled SV40 FO I DNA (15 000 cpm, 0.09 μg) and 10 μl S_1 enzyme in 30 mM sodium acetate, 50 mM NaCl, 1 mM Zn^{2+}, 5% glycerol, pH 4.6. The reaction was carried out for 30 min at 37°C. (• – •) fd DNA; (○ – ○) SV40 DNA. (B) 30 μl (2000 cpm, 0.07 μg) ^{32}P fd RF. Arrows show the positions of standard markers run in parallel tracks of the same gel.

II generated fd RF form III marker (Hind II cleaves fd RF only once [9]). Part of the fd RF molecules remained unattacked by S_1 nuclease and migrated slower than form III (for comparison see fig.2b, where the position of untreated fd RF is depicted).

It should be emphasized that the extent to which SV40 superhelical DNA was converted to form III (approximately 70% of input) is only slightly larger than the relative amount of fd RF form III (approximately 60% of input). Thus, it is clear from this result that superhelical configuration is neither a prerequisite nor is it advantageous for the double-strand cleaving activity in S_1 nuclease preparations.

The S_1-generated fd RF form III product was analysed by sedimentation through an alkaline sucrose gradient. Most of the material sedimented as a homo-

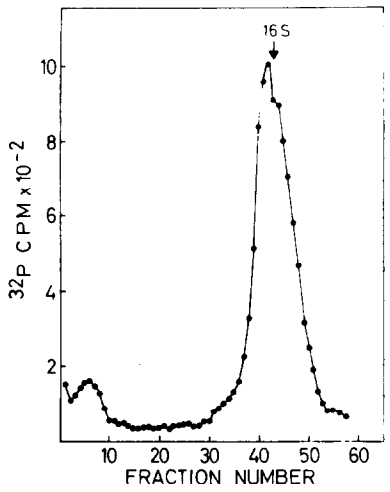

Fig.3. Alkaline velocity analysis of S_1-treated ^{32}P fd RF DNA. The reaction mixture (218 μl) contained 175 μl ^{32}P fd RF (11580 cpm, 0.41 μg), 10 μl ^{3}H-labeled SV40 FO I (23 000 cpm, 0.14 μg) and 25 μl S_1 enzyme. Incubation took place for 60 min at 37°C in S_1 buffer. The reaction was terminated by addition of 20 μl of 0.2 M EDTA. The arrow indicates the position of S_1 generated ^{3}H-labeled SV40 form III DNA.

geneous peak somewhat faster than the SV40 form III marker. Some residual fd RF which had not been cleaved open by S_1 nuclease (see fig.2) appeared as fast sedimenting supercoiled DNA at the bottom of the gradient. These results show that conversion of the circular fd RF to open form III of unit length by S_1 nuclease occurs by introduction of one double-strand break into the DNA.

The data reported above have extended our previous results [5] where form III appeared as reaction product after treatment of relaxed multi-nicked SV40 DNA form II with S_1 nuclease. Here we show that this conversion of form II to form III by S_1 nuclease can occur in the absence of both single-strand nicks and topological constraints. We have suggested [4] that S_1 nuclease resembles with regard to its action on circular DNA some complex type bacterial restriction nucleases which attack circular DNA (at random sites) and, which also cleave each DNA molecule only once [10,11]. The mechanism by which only one double-strand cleavage per molecule of double-stranded DNA is brought about by these enzymes is unclear and awaits further elucidation.

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References

[1] Méchali, M., de Recondo, A. M. and Girard, M. (1973) Biochem. Biophys. Res. Commun. 54, 1306–1320.
[2] Beard, P., Morrow, J. F. and Berg, P. (1973) J. Virol. 12, 1303–1313.
[3] Germond, J., Vogt, V. and Hirt, B. (1974) Eur. J. Biochem. 43, 591–600.
[4] Waldeck, W., Chowdhury, K., Gruss, P. and Sauer, G. (1976) Biochim. Biophys. Acta 425, 157–167.
[5] Chowdhury, K., Gruss, P., Waldeck, W. and Sauer, G. (1975) Biochem. Biophys. Res. Commun. 64, 709–716.
[6] Heyden, B., Nüsslein, C. and Schaller, H. (1972) Nature New Biology, 240, 9–12.
[7] Waldeck, W., Kammer, K. and Sauer, G. (1973) Virology 54, 452–464.
[8] Shenk, T. E., Rhodes, C., Rigby, P. W. and Berg, P. (1975) Proc. Natl. Acad. Sci. USA 72, 989–993.
[9] Tabak, H. F., Griffith, J., Geider, K., Schaller, H. and Kornberg, A. (1974) J. Biol. Chem. 249, 3049–3054.
[10] Adler, S. P. and Nathans, D. (1973) Biochim. Biophys. Acta 299, 177–188.
[11] Morrow, J. F. and Berg, P. (1972) Proc. Natl. Acad. Sci. USA 69, 3365–3369.