A STUDY ON THE CLEAVAGE SITE SPECIFICITY OF THE ENDONUCLEASE ASSOCIATED WITH PURIFIED SIMIAN VIRUS 40 PARTICLES

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1. Introduction

Purified simian virus 40 (SV40) particles contain an endonucleolytic activity which can convert SV40 component I DNA (a closed circular, double stranded, superhelical molecule) into a nicked form [1, 2]. The capsid protein responsible for this activity has not been identified and it is unclear whether the enzyme is coded for by the host or by the viral genome. The enzyme is present in virions purified from several different cell lines and in three temperature-sensitive mutants of SV40 [2]; a temperature-sensitive mutant of the closely related polyoma virus has been shown to be defective in this activity [3]. Recent studies have indicated that an endonuclease is probably involved in the replication of SV40 DNA [4, 5] and in the recombination between SV40 DNA and cellular DNA [6–8]. Clearly, the specificity of cleavage of DNA by the SV40-associated endonuclease is of interest. In this report I describe experiments designed to determine if the cleavage of bacteriophage λ DNA by this endonuclease occurred at specific or random nucleotide sequences. The nucleotides produced by digestion with this enzyme were heterogeneous and terminal labelling studies with polynucleotide kinase indicated that cleavage was random. It was therefore concluded that the SV40-associated endonuclease is a non-specific endonuclease.

2. Materials and methods

2.1. *SV40 and λDNA preparation*

Monolayers of MA134 cells were infected with SV40 strain 777 (three times plaque purified; obtained from Dr. H. Westphal, National Institutes of Health, Bethesda, Maryland) at a multiplicity of 5–10 plaque forming units/cell. Cultures were grown until lysis and SV40 was purified as described previously [9]. The virus was stored frozen at −20°C; electrophoretic analysis on polyacrylamide gels showed that the virus preparations were free of detectable host protein contamination. Bacteriophage λ was isolated from lysogens of *E. coli* strain M5107 [from Dr. E.R. Signer via Dr. E. Bade, Cold Spring Harbor Laboratory; genotype *E. coli* K-12 F^- su^- $gal^=$ str^R T_1^R λ^R(λcI857 S7)] as described by Goldberg and Howe [10]. Cultures were sometimes labelled with [^{32}P]inorganic phosphate (10 μCi/ml of culture) during the induction at 42°C. The bacteriophage was purified by equilibrium density centrifugation in caesium chloride and the DNA isolated by phenol extraction [11].

2.2. *Digestion with the SV40-associated endonuclease*

DNA was dissolved in 6.6 mM Tris–HCl, pH 7.5, 50 mM NaCl, 6.6 mM β-mercaptoethanol, 6.6 mM $MgCl_2$ (normally 100 μl) and was digested at 37°C with the SV40-associated endonuclease (enzyme substrate ratio, 5 μg of purified virus protein/μg of λ DNA). After digestion, the solutions were extracted once with freshly distilled phenol and twice with ether. Material that was to be phosphorylated with polynucleotide kinase was normally digested with bacterial alkaline phosphatase (1 mg/ml in 0.1 M NH_4HCO_3, enzyme substrate ratio approx. 1 μg enzyme/μg of DNA) at 37°C for 60 min; the phosphatase was subsequently extracted with phenol.

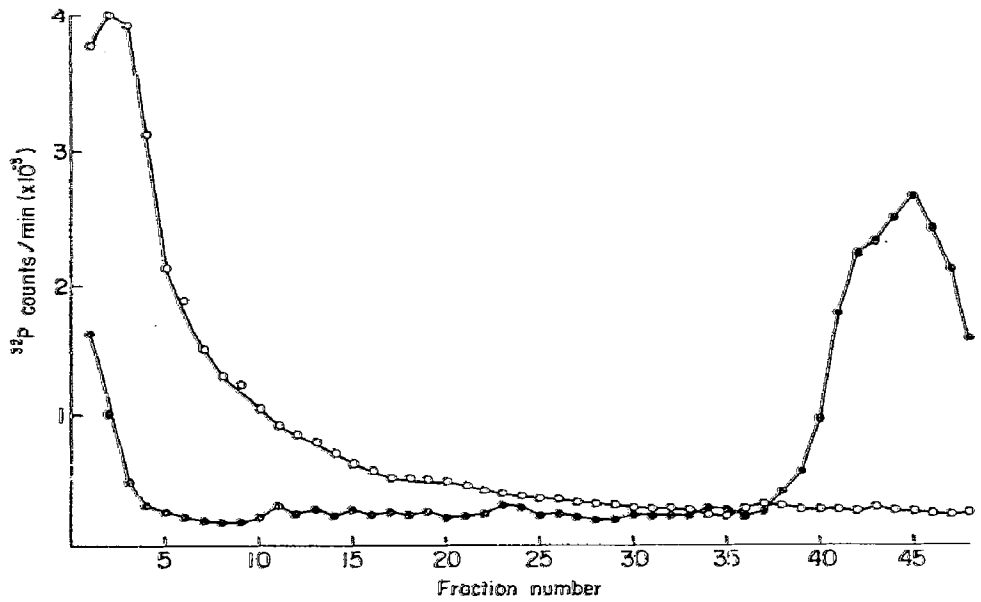

Fig. 1. Velocity sedimentation of the SV40-associated endonuclease digestion products of λ DNA. [^{32}P]λ DNA was digested with the SV40-associated endonuclease at 37°C for 30 min. Digestion was stopped by the addition of 0.3 M NaOH (200 μl) and the products were separated by centrifugation through a 5–20% alkaline sucrose gradient (5–20% sucrose in 0.3 M NaOH, 2 mM EDTA) at 40 000 rev/min for 3.5 hr at 20°C in a Spinco SW50.1 Rotor. (o—o—o) Undigested λ DNA. (•—•—•) Digested λ DNA.

2.3. *Terminal labelling, nuclease digestion and ionophoresis*

Polynucleotide kinase was prepared from bacteriophage T_4-infected *E. coli* [12] and was used according to Murray [13]. 5–10 μg of digested λ DNA in 6.6 mM Tris–HCl pH 7.5, 6.6 mM β-mercaptoethanol, 6.6 mM $MgCl_2$ was incubated with 100 μCi ATP (γ-^{32}P-labelled; specific radioactivity 5–15 Ci/mmol) and polynucleotide kinase (5 units) at 37°C for 4 hr. The reaction was stopped either by phenol extraction or by heating at 100°C for 2 min. Digestion of either terminally labelled or uniformally labelled material with pancreatic deoxyribonuclease and snake venom phosphodiesterase was as described by Murray [11]. Fractionation of radioactive oligonucleotides was by ionophoresis on either cellulose acetate at pH 3.5 or DEAE-cellulose paper at pH 9.5 in the first dimension followed by ionophoresis on DEAE-cellulose paper at pH 2 in the second [11, 14]. Mononucleotides were separated by ionophoresis on AE-cellulose paper at pH 3.5 [11]. Nucleotides were detected radioautographically on Kodak blue-brand medical X-ray film and were quantitated, when required, by liquid scintillation counting.

3. Results and discussion

The degradation of λ DNA by the SV40-associated endonuclease was initially analyzed by velocity sedimentation through alkaline sucrose gradients (fig. 1). The digestion products were relatively small, sedimenting as a broad peak at less than 7–10 S; observations under the electron microscope showed that these polynucleotides had a heterogeneous length distribution. The two-dimensional ionophoretic separation of this digest (fig. 2) confirmed the heterogeneity of the oligonucleotides that were small enough to be resolved by this technique. Mononucleotides were detected during digestion with the SV40-associated endonuclease only after prolonged incubation (> 4 hr). Non-specific endonucleases are known to eventually produce small amounts of mononucleotides however the presence of a contaminating exonuclease, although unlikely, can not be excluded.

Polynucleotide kinase labelling of the nucleotides produced by digesting λ DNA with the SV40-associated endonuclease for different times is shown in fig. 3. Efficient labelling of these digests was observed only after treatment with phosphatase and hence the

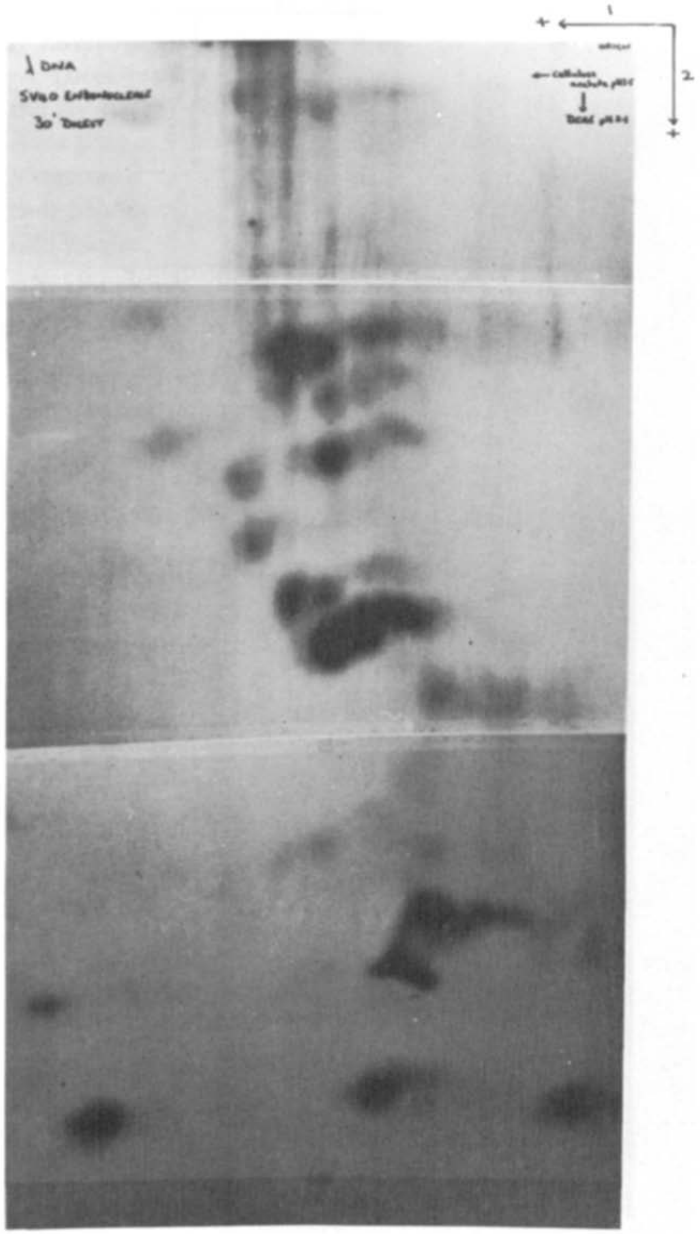

Fig. 2. Separation of the nucleotides produced by digesting [^{32}P]λ DNA with the SV40-associated endonuclease. Fractionation was by ionophoresis on cellulose acetate at pH 3.5 in the first dimension and on DEAE-cellulose paper at pH 2 in the second.

terminal nucleotides predominantly contained 5-phosphoryl groups. The limit digest was not observed during these experiments.

Specificity in the cleavage of λ DNA by the SV40-associated endonuclease was investigated by isolating

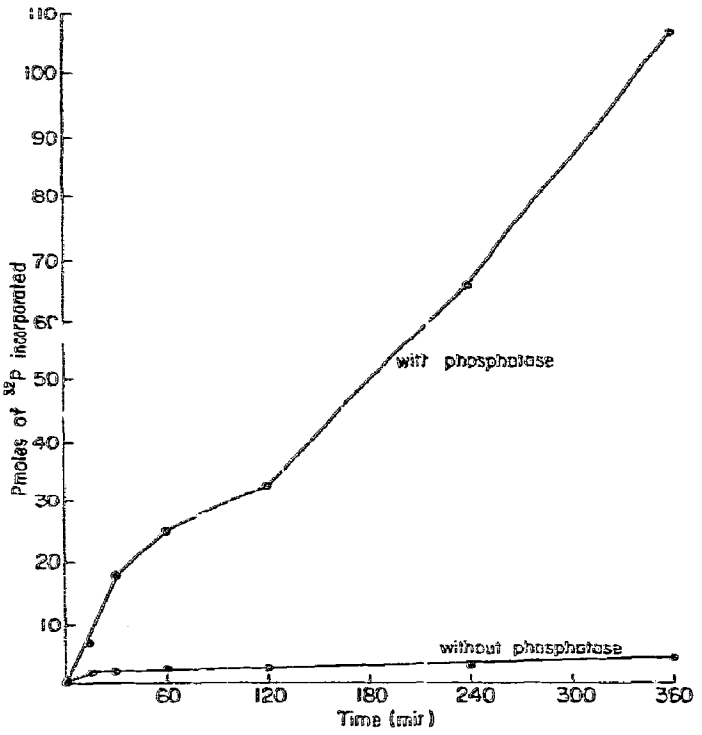

Fig. 3. Polynucleotide kinase labelling of SV40-associated endonuclease digests of λ DNA. One set of digests were treated with phosphatase before labelling. The phosphorylated products were precipitated with 0.1 M perchloric acid at 0°C for 30 min and the precipitates subsequently collected by centrifugation. The precipitates were dissolved in 0.2 M NaOH and precipitation and dissolution repeated a further three times. After the final precipitation, the precipitates were collected on glass fibre discs and counted in a liquid scintillation spectrometer.

Table 1

The 5'-terminal mononucleotides of the degradation products of λ DNA.

Digestion time	0 (min)	15	60	120
Nucleotide				
dGMP	52.7	18.9	23.6	23.6
dAMP	34.3	29.2	25.6	26.6
dCMP	5.5	24.0	23.4	23.8
dTMP	7.5	27.9	27.4	25.9
Total ^{32}P incorporated (pmole)	0.05	18.6	24.7	33.7

Mononucleotides were recovered after the digestion of terminally labelled material with pancreatic deoxyribonuclease and snake venom phosphodiesterase (Materials and methods). These results are expressed as mol/100 mol of recovered mononucleotides and are corrected for the small amounts of ^{32}P incorporated into the 5'-terminal mononucleotides of undigested λ DNA (0 time).

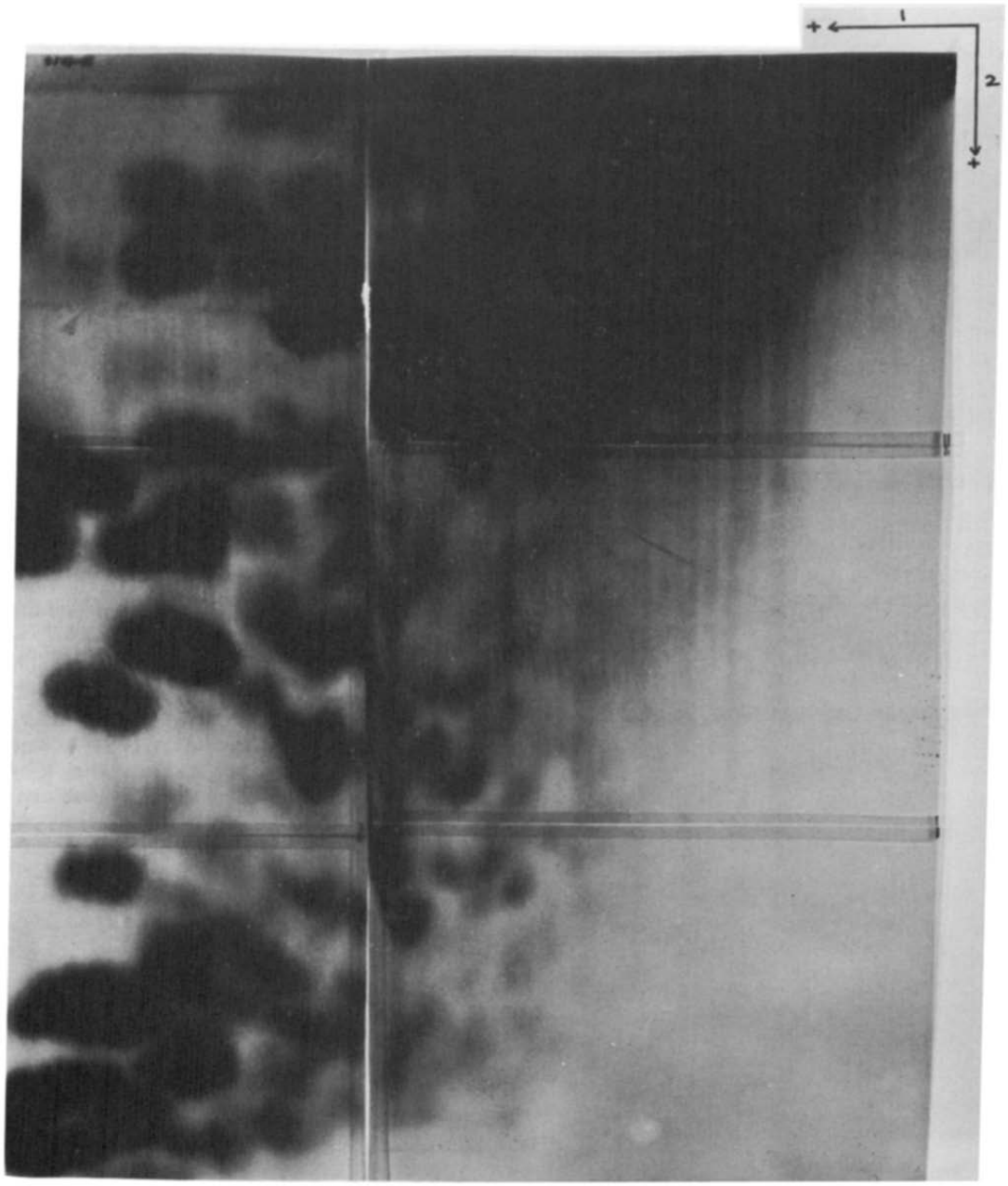

Fig. 4. Terminally labelled nucleotides from an SV40-associated endonuclease digest of λ DNA. λ DNA was digested with this endonuclease at 37°C for 6 hr and the products labelled by reaction with polynucleotide kinase after treatment with phosphatase. The phosphorylated material was subsequently digested with pancreatic deoxyribonuclease and the terminal oligonucleotides separated by ionophoresis on DEAE-cellulose paper at pH 9.5 in the first dimension and at pH 2 in the second.

the 5'-terminal mono- and oligonucleotides after labelling the cleavage products with polynucleotide kinase. Concomitant labelling of all four terminal mononucleotides was observed even after short periods of digestion with the SV40-associated endonuclease (table 1). The 5'-terminal oligonucleotides, fractionated

by two-dimensional ionophoresis, were correspondingly heterogeneous (fig. 4).

These results show that digestion of λ DNA with the SV40-associated endonuclease produces nucleotides that are relatively small and heterogeneous. The 5'-terminal mono- and oligonucleotides of the digestion products, isolated after labelling with polynucleotide kinase, were also shown to be heterogeneous. It was concluded that the SV40-associated endonuclease is not highly specific with respect to the nucleotide sequence at which it cleaves. This report does not however rule out the possibility that purified SV40 particles contain more than one nucleolytic activity.

Acknowledgements

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