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Processive action of T4 endonuclease V on ultraviolet-irradiated DNA

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ABSTRACT

The action of the dimer-specific endonuclease V of bacteriophage T4 was studied on UV-irradiated, covalently-closed circular DNA. Form I ColE1 DNA preparations containing average dimer frequencies ranging from 2.5 to 35 pyrimidine dimers per molecule were treated with T4 endonuclease V and analysed by agarose gel electrophoresis. At all dimer frequencies examined, the production of form III DNA was linear with time and the double-strand scissions were made randomly on the ColE1 DNA genome. Since the observed fraction of form III DNA increased with increasing dimer frequency but the initial rate of loss of form I decreased with increasing dimer frequency, it was postulated that multiple single-strand scissions could be produced in a subset of the DNA population while some DNA molecules contained no scissions. When DNA containing an average of 25 dimers per circle was incubated with limiting enzyme concentrations, scissions appeared at most if not all dimer sites in some molecules before additional strand scissions were produced in other DNA molecules. The results support a processive model for the interaction of T4 endonuclease V with UV-irradiated DNA.

INTRODUCTION

The endonuclease V purified from Escherichia coli infected with T4 has been shown to specifically incise strands of DNA containing pyrimidine dimers (1-3). Although it was previously thought that these incisions resulted from cleavage of a phosphodiester bond on the 5' phosphate side of each dimer (1) recent studies have indicated a more complex, two-step reaction (4-6). It appears that the first step in incision is cleavage of the N-glycosylic bond between one of the dimerized pyrimidines and its sugar, and the second step involves the cleavage of the phosphodiester bond between the pyrimidines.

Previously Friedberg and Clayton (7) observed no linear SV40 DNA molecules by electron microscopy after form I SV40 DNA containing less than 2 pyrimidine dimers per molecule was treated with endonuclease V. In addition, Simon et al. (3) found that endonuclease V does not cleave the DNA strand opposite a dimer, thus demonstrating that the enzyme does not directly produce

double-strand scissions. Minton and Friedberg (8) reported endonuclease V-mediated double-strand scissions in T4 and T7 DNA irradiated with 20 and 53 J/m^2 respectively and attributed these observations to dimer clustering at high UV doses. However, this conclusion should possibly be reconsidered in light of a significant amount of evidence which argues against clustering of thymine dimers in UV-irradiated DNA (9-11). We have observed a large proportion of linearized DNA molecules after treatment of superhelical DNA with T4 endonuclease V. Further, we document that these double-strand scissions arise by a processive enzymatic action of the T4 endonuclease V.

MATERIALS AND METHODS

DNA preparation

ColE1 DNA was prepared by a modification of the procedure of Clewell and Helinski (12). *E. coli* AB2487 (F^- λ^S *recA13 leu thr thi arg pro his thyA thyR lac ara gal mtl xyl*) was originally received from Ray Rodriquez (13). An overnight culture was grown at 37° in Davis minimal broth (Difco) (10.6 g/l) supplemented with 0.4% glucose, 1 mM each of leucine, arginine, proline, histine, 0.05% casein hydrolysate, 0.5 µg/ml vitamin B1 and 10 µg/ml thymine. It was diluted 1:100 (into 200 ml) and 2 µCi of [^{3}H]-thymine or 100 µCi of [^{14}C]-thymine was added. In late exponential phase (8×10^8 cells/ml) chloramphenicol was added to 150 µg/ml and incubation was continued for 14 hr. Cells were chilled to 0°C and pelleted at 4,500 rpm in a Sorvall type SS-34 rotor, washed with 5 ml of 10 mM Tris, pH 8.0, 1 mM EDTA, pelleted and resuspended in 2.5 ml of 50 mM Tris, pH 8.0, 25% sucrose. Suspensions were incubated with lysozyme (2 mg/ml) for 10 min at 0°C, and EDTA was added to 50 mM. After 5 more min at 0°C, an equal volume of 10 mg/ml Brig 58, 4 mg/ml sodium deoxycholate, 50 mM EDTA, 50 mM Tris, pH 8.0, was added. After 20 min at 23°C the lysate was centrifuged at 12,500 rpm in a Sorvall type SS-34 rotor for 1 hr at 20°C. The supernatant, containing the ColE1 DNA, was collected and solid CsCl was added (1 gm per ml of supernatant), and dissolved. Ethidium bromide was added to 1 mg/ml. After 40 hr centrifugation at 37,000 rpm in an SW50.1 rotor, the lower DNA band was collected and the ethidium bromide removed by extraction with CsCl saturated isopropanol. The DNA was dialyzed twice against 2 liters of 10 mM Tris, 10 mM NaCl, 1 mM EDTA, pH 7.8.

Enzymes

The T4 endonuclease V was purified exactly by the procedure of Seawell et al. (14). The endonuclease V preparation (2 mg/ml of protein) contained 910 nicking units of activity per µl. One unit is defined as the amount of

enzyme that nicks 1 fmole of ColE1 DNA irradiated with 20 J/m^2 at 254 nm in 1 min at 37°. The restriction enzymes EcoRI and PstI were purchased from New England Biolabs.

UV-irradiation

Purified ColE1 DNA (28-230 µg/ml) 1-2 mm deep in depressions of a glass palette was irradiated using an unfiltered 15 watt germicidal lamp. The incident dose rate was measured by an IL 254 Germicidal Photometer (International Light, Newburyport, Mass.). The pyrimidine dimer content in ColE1 DNA after irradiation with ten selected UV doses between 0 and 300 J/m^2 was determined by thin layer chromatography on silica gels (15). Assuming a thymine content of 0.25 (16), a pyrimidine dimer ratio of 59:34:7 for $\hat{T}\hat{T}$:$\hat{T}\hat{C}$+$\hat{C}\hat{T}$:$\hat{C}\hat{C}$ (17) and a genome size of 4.2×10^6 daltons, a value of 0.09 dimer per $J \cdot m^{-2}$ per ColE1 was calculated from the percent thymine in dimers (P. C. Seawell, J. C. Hunt, and A. K. Ganesan, personal communication).

Reactions with T4 endonuclease V

For standard reactions 275 nicking units (7.5 µl) of T4 endonuclease V in 30 mM Tris, 6 mM NaCl, 0.6 mM EDTA, 0.6 mg/ml BSA 0.25% (w/v) ethylene glycol was added to 150 µl of 57 µg/ml ColE1 containing 0-35 dimers per molecule in 50 mM Tris, pH 8.0, 10 mM NaCl, 1 mM EDTA. All reactions were incubated at 37°. Those which were to be analyzed by agarose gel electrophoresis were terminated by the addition of an equal volume of 50 mM Tris pH 8.0, 1 mM NaCl, 0.1 mM EDTA, 1% (w/v) sarkosyl, 20% (w/v) sucrose and 0.0125% (w/v) bromphenol blue. Reactions which were to be analyzed on sucrose gradients were terminated by the addition of 1/10 volume of 10% sarkosyl.

Forms I, II, and III (superhelical, nicked circular and linear forms, respectively) of the DNA were resolved by electrophoresis through 1% (w/v) agarose tube gels (Biorad agarose powder) for 2.5 hr at 100 V. The gels were stained with 1 µg/ml ethidium bromide in electrophoresis buffer (40 mM Tris, 33 mM sodium acetate, 18 mM NaCl, 2 mM EDTA, pH 8.2). The positions of the three DNA bands were determined by visualization with long wavelength UV light and slices of the gel 3-5 mm in length containing the DNA bands were cut. Each slice was placed in a glass vial and after the addition of 0.1 ml of 1.0 N HCl, the vials were covered with aluminum foil and briefly heated to 100°C. After cooling, 3 ml of Packard Insta-gel was added to each and the radioactivity determined by liquid scintillation spectrophotometry.

DNA was also analysed by centrifugation on alkaline sucrose gradients (5-20% w/v sucrose in 0.1 N NaOH) for 5 hr at 45,000 rpm at 20°C in a Beckman

SW50.1 rotor. Fractions were collected on strips of Whatman #17 filter paper which were washed, dried, cut and assayed for radioactivity (18). The number average molecular weight (Mn) was calculated from the profiles as described by Rupp and Howard-Flanders (19), using form III ColE1 DNA as the standard. Fractions containing at least 5% of the total radioactivity in the gradient were utilized for calculating the Mn values. The frequency of nicks in circular DNA equals (Mn of unirradiated control/Mn irradiated sample).

Preparation of DNA for restriction enzyme analysis

ColE1 DNA (57 µg/ml in 50 mM Tris, 10 mM NaCl, 1 mM EDTA pH 8.0) which contained 25 dimers per molecule was incubated with T4 endonuclease V (275 nicking units) in a total reaction volume of 0.315 ml for 10 min at 37°C. The reaction was terminated by the addition of sodium acetate to a final concentration of 0.4 M and 1 ml of 95% ethanol. After 12 hr at -20°C, the DNA was collected by centrifugation, washed with 70% ethanol and resuspended in 5 mM Tris, 1 mM NaCl, 0.1 mM EDTA, pH 7.8. Five units of either PstI or EcoRI restriction enzyme were used in the reaction buffers suggested by the supplier. Digestions were terminated by the addition of an equal volume of 50 mM Tris, 20 mM EDTA, 10% (w/v) sucrose, 0.0125% (w/v) bromphenol blue, pH 7.8. Following electrophoresis through a 1% agarose vertical slab gel, the DNA was stained with 1.0 µg/ml ethidium bromide in electrophoresis buffer. The lanes of interest were cut out and sliced into 2mm pieces. Slices were assayed as previously described.

RESULTS

Strand breaks produced as a function of dimer concentration

When DNA containing an average of 25 dimers per molecule was exposed to the preparation of T4 endonuclease V, full-length linear molecules (form III) were produced and the percent of DNA as form III increased linearly from 0 initially to 23 percent after 20 min (Fig. 1). Form I DNA decreased from 88 percent of the population at time zero to 6 percent after 20 min of exposure to the enzyme.

The production of form III molecules by the enzyme preparation was then examined with DNA substrates containing several different pyrimidine dimer frequencies (Fig. 2). As the dimer concentration in the substrate increased, the rate of accumulation and the amount of form III DNA produced increased. The production of form III DNA was linear with time for each substrate DNA throughout these experiments and the rate of production was directly proportional to the dimer content of the DNA. Thus as the average number of dimers

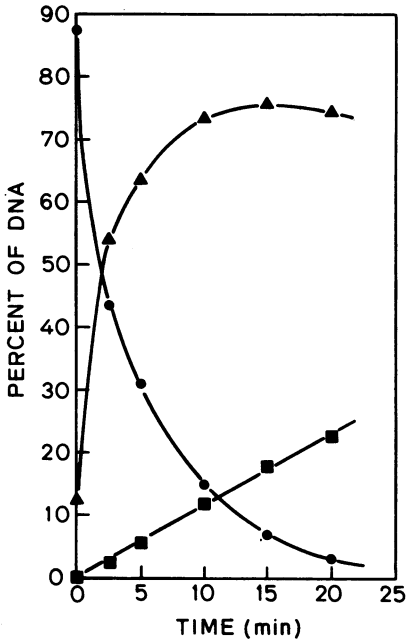

Fig. 1. Production of single- and double-strand scissions by T4 endonuclease V in UV-irradiated ColE1 DNA containing 25 dimers per molecule. A mixture of forms I and II ColE1 DNA (57 μg/ml) was treated with 275 nicking units of T4 endonuclease V as described in Materials and Methods and the DNA reaction products were determined as a function of incubation time: ●, form I DNA; ▲, form II DNA; ■, form III DNA.

per molecule was increased by a factor of 2, 3, or 5 times, the observed percent form III DNA also increased by that factor, respectively. Although the rate of accumulation of form III DNA increased as a function of the average number of dimers per DNA molecule, the rate at which form I DNA was converted to either forms II or III DNA decreased (Fig. 2).

The rate of loss of form I DNA and the accumulation of form III DNA were also investigated as a function of DNA concentration using substrate DNA containing an average of 25 dimers per molecule and a constant amount of enzyme (Fig. 3). The rate of loss of form I DNA was reduced as the DNA concentration was increased from 28 μg/ml to 230 μg/ml. Within the range of the DNA concentrations used, any increase in the DNA concentration resulted in a corresponding decrease in the rate of loss of form I DNA. This demonstrated that the enzyme concentration was limiting in all reactions. The fraction of molecules converted to form III was a linear function of time for all DNA concentrations examined, but was significantly reduced as the concentration of DNA was

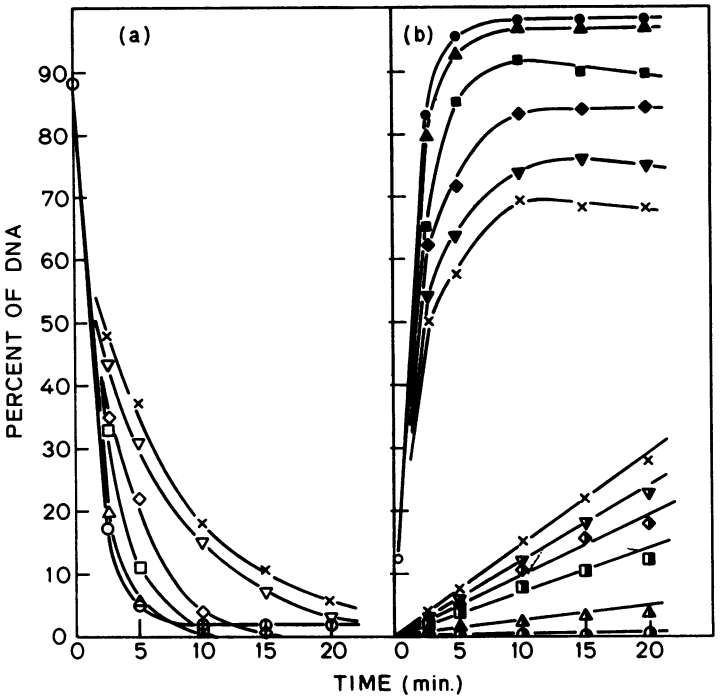

Fig. 2. T4 endonuclease V-induced strand scission as a function of the pyrimidine dimer concentration in the substrate DNA. ColE1 DNA (57 μg/ml) containing different average numbers of dimers per molecule was treated with 275 nicking units of T4 endonuclease V as described in Materials and Methods. (a) Percent of DNA as form I; (b) percent of DNA as form II (filled symbols) and form III (half-filled symbols). Similarly shaped symbols show data for substrate DNA containing the following average number of dimers per molecule: ○, 2.5; △, 5.0; □, 10; ◇, 15; ▽, 25; X, 35.

increased. However, this difference was expected because as the ratio of DNA molecules to enzyme molecules increased, more breakage events must have occurred to change the percentages of DNA in forms I, II and III to an equal degree. Thus when the DNA was in excess, the total number of DNA breakage events per unit time was simply dependent upon the number of enzyme molecules.

In order to determine whether these double-strand scissions were produced randomly or at specific locations on the DNA, ColE1 DNA (57 μg/ml) containing an average of 25 dimers per molecule was incubated with T4 endonuclease V for 10 min at 37°C. After repurification, it contained 70% form II and 30% form III DNA. Separate portions of this DNA were then treated with the restriction endonucleases EcoRI or PstI, which have one and two cleavage sites respectively on ColE1 DNA. If the endonuclease V-produced double-strand breaks were

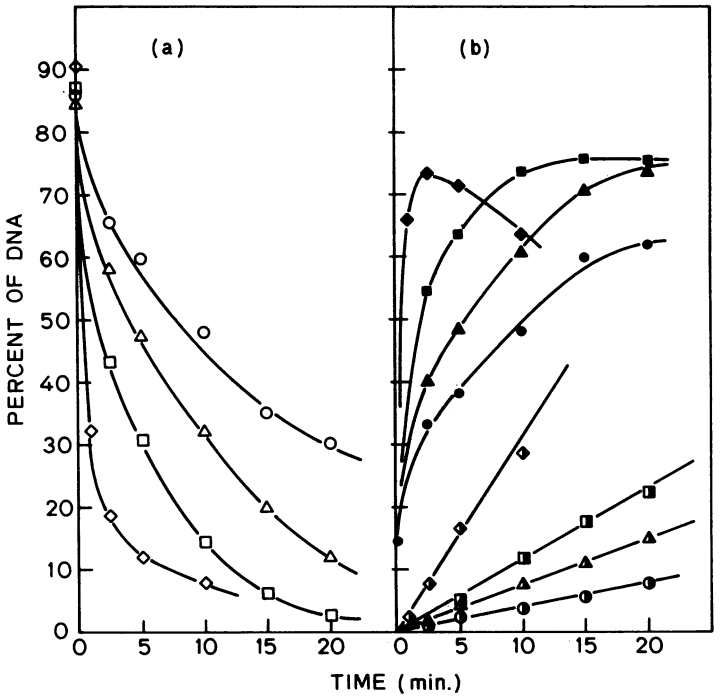

Fig. 3. DNA strand scission by T4 endonuclease V as a function of DNA concentration. The change in the percentage of forms of ColE1 DNA which contained an average of 25 dimers per molecule was followed as a function of both the time of incubation with the enzyme and the concentration of DNA in the reaction mixture. (a) Percent of DNA as form I; (b) percent of DNA as form II (filled symbols) and form III (half-filled symbols). Similarly shaped symbols show the data for the different DNA concentrations: ○, 230 μg/ml; △, 115 μg/ml; □, 57 μg/ml; ◇, 28 μg/ml.

at specific sites, the restriction enzyme digest would be expected to contain DNA fragments of specific lengths corresponding to the distances between these sites and the sites of restriction cleavage. These DNA fragments should then be seen as specific bands after agarose gel electrophoresis. However, if the sites of double-strand cleavage were not at specific locations on the DNA, a smearing of DNA migrating in front of DNA restriction fragments would be expected since some of the fragments would have been reduced in length in random fashion. The latter result was obtained (Fig. 4). No new specific bands were detected, but smearing of the DNA ahead of the position of the major restriction fragments was clearly observed. Although this result does not rule out the possibility that some nucleotide sequences in the DNA could more readily form dimers than other sequences, it indicates that the distribution

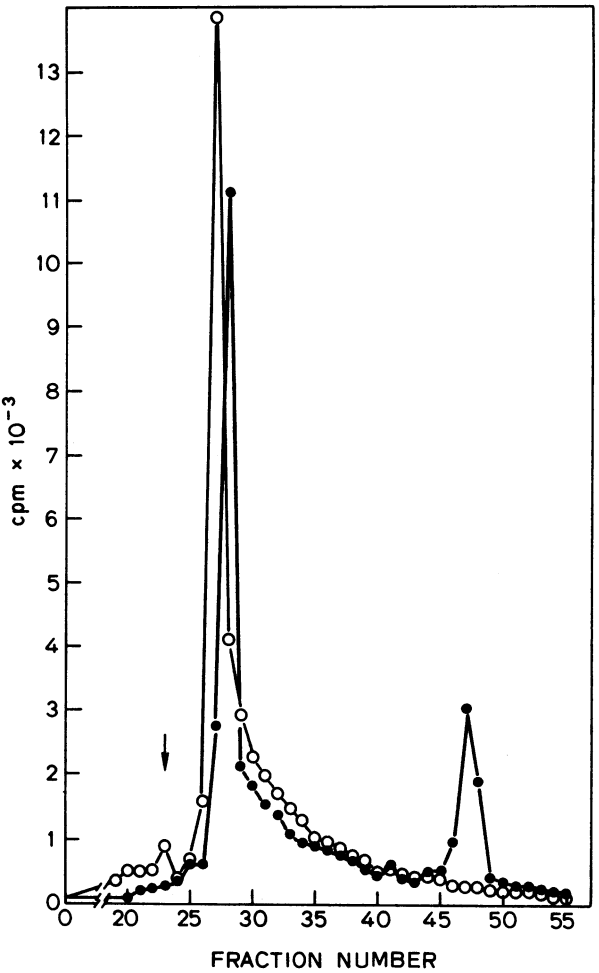

Fig. 4. Random production of double strand breaks produced by T4 endonuclease V in the ColE1 DNA genome. ^{3}H ColE1 DNA (90 percent form I) containing an average of 25 dimers per molecule was treated with the enzyme to produce 70% form II and 30% form III DNA. The DNA was repurified and treated with either EcoRI or PstI restriction endonuclease. Following electrophoresis (migration is to the right) the amount of radioactivity was determined in 2 mm slices of the gels: O, EcoRI-treated ColE1 DNA; ●, PstI-treated ColE1 DNA. The arrow indicates the position of form II ColE1 DNA.

of lesions in DNA that lead to double-strand scissions by endonuclease V was indistinguishable from a random distribution.

Distribution of lesions in the DNA population

T4 endonuclease V clearly produces a significant percentage of form III

DNA early in the reaction. In order to determine whether the appearance of form III is due to an accumulation of random single-strand scissions to produce closely spaced single-strand scissions in complementary DNA strands, we applied the equations of Freifelder and Trumbo (20) to our data. In this analysis one assumes a Poisson distribution of single-strand breaks (estimated by the negative natural logarithm of form I DNA) and that a double-strand break would be produced by two single-strand breaks within a distance h base pairs of one another on complementary DNA strands. Under the ionic and temperature conditions used, h should equal no more than 12-16 base pairs (20). Using these assumptions, for DNA containing an average of 35, 25, and 15 dimers per molecule treated with enzyme for 5 min (Fig. 2), the percentages of form III DNA calculated from these equations would be 0.6, 0.9, and 1.5 respectively. However, the observed percentages of form III DNA were 7.8, 5.9 and 5.0 (Fig. 2). Thus, using these assumptions it is impossible to account for the large accumulation of form III DNA simply by a random accumulation of single-strand breaks. However, it might be explained by production of multiple single-strand scissions within a subset of the DNA molecules while no breaks were produced in other DNA molecules.

This possibility was tested by analyzing the number of nicks in the DNA as determined by its number average molecular weight after enzyme treatment. Separate DNA samples containing an average of 2.5 and 25 pyrimidine dimers per molecule were incubated with endonuclease V under conditions predicted to leave 20% of the molecules as form I (275 nicking units for 2.5 or 10 min, respectively). Analysis on neutral agarose gels indicated that 19% and 15% form I remained for the two cases, respectively. A Poisson analysis predicts about 2 single-strand breaks per molecule in both populations. Velocity sedimentation through alkaline sucrose gradients showed that in the case of the DNA containing 2.5 dimers per molecule, most of the molecules that were not form I contained 2 single-strand breaks. However, in the case of the DNA containing 25 dimers per molecule, most of the molecules which were not form I contained many more than 2 single-strand breaks (Fig. 5). Values for $\bar{M}_n$ calculated from these profiles indicated approximately 22 single-strand scissions per molecule. Thus the actual number of single-strand scissions was not accurately predicted for highly irradiated DNA by applying the Poisson analysis to the remaining percentage of form I DNA. Analysis of identical reactions containing only half the previous amount of enzyme showed that the relative amount of DNA in each peak was altered but the positions of the peaks in the gradients were not changed. These data support the hypothesis

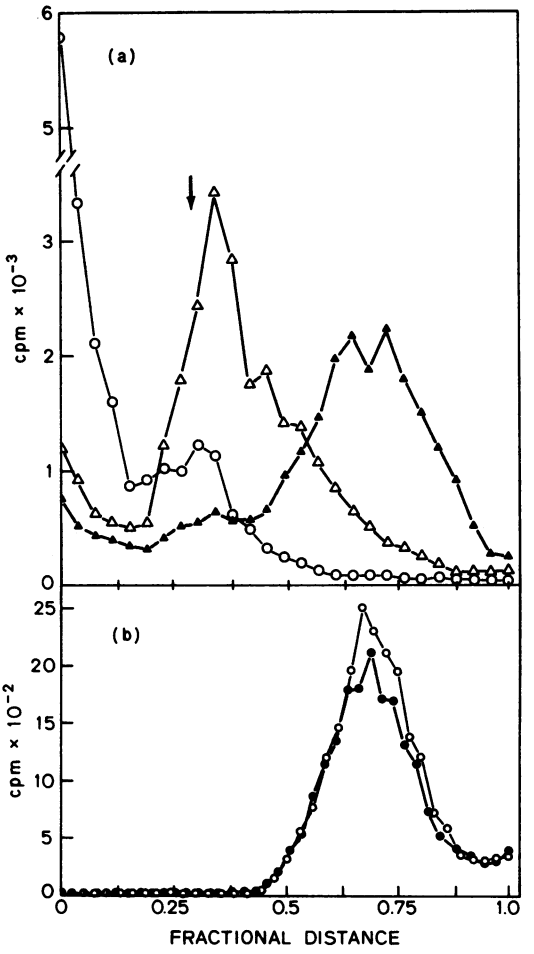

Fig. 5(a) Sedimentation analysis on alkaline sucrose gradients of the products of DNA-endonuclease V reactions. The DNA contained either 2.5 or 25 dimers per molecule and the reaction was terminated at different times such that approximately 20% of the DNA remained as form I. Analysis on agarose gels indicated that the percentages of forms I, II and III were actually 74, 25.4 and 0.6 for untreated DNA; 19, 80, and 0.9 in the DNA which contained an average of 2.5 dimers per molecule (incubated for 2.5 min with 275 nicking units of endonuclease V); and 15, 71.4 and 13.6 for the DNA which contained 25 dimers per molecule (incubated 10 min with 275 nicking units of endonuclease V). Sedimentation was from the right to left. ○, control [^{3}H]-ColE1 DNA not reacted with endonuclease V; △, [^{3}H]-ColE1 DNA (2.5 dimers per molecule) after 2.5 min of reaction with endonuclease V; ▲, [^{3}H]-ColE1 DNA (25.0 dimers per molecule) after 10 min of reaction with endonuclease V. The arrow indicates the position unit length linear [^{14}C]-ColE1 DNA. (b) Limit digestion of ColE1 DNA containing 25 dimers per molecule with either ○, 13,750 nicking units or ●, 27,500 nicking units of T4 endonuclease V for 60 min. Sedimentation was as in (A).

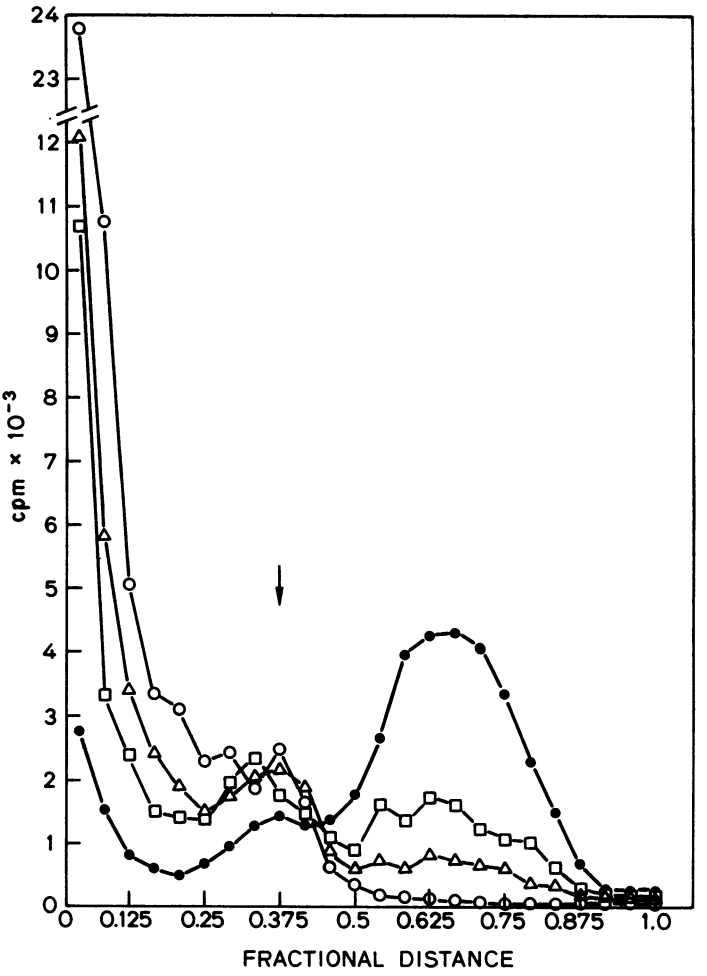

Fig. 6. Intermediate fragmentation of ColE1 DNA containing 25 dimers per molecule upon exposure to T4 endonuclease V. [^{14}C]-ColE1 DNA (57 µg/ml) containing 25 dimers per molecule was incubated with the enzyme (137.5 nicking units) as described in the Materials and Methods for the following times: ○, zero min; △, 5 min; □, 15 min; and ●, 60 min. Sedimentation through a 5-20% (w/v) alkaline sucrose gradient was from right to left. The arrow indicates the position of EcoRI-cut full length linear [^{3}H]-ColE1 DNA. Agarose gel electrophoresis revealed the following percentages of forms I, II and III DNA respectively at the indicated times of reaction: 0 min, 88.6, 11.4 and 0; 5 min, 71.8, 26.3 and 1.9; 15 min, 53.1, 43.3 and 3.6; and 60 min, 25.4, 63.0 and 11.6.

that when a molecule of T4 endonuclease V nicks a DNA molecule at the site of a pyrimidine dimer, it then nicks the remaining dimer sites on that molecule before it proceeds to other DNA molecules.

In order to verify that the size of the single-stranded DNA (Fig. 5, closed triangles) was indicative of incision at all endonuclease V sensitive sites on some of the molecules, ColE1 DNA containing 25 dimers per molecule was treated with 13,750 and 27,500 nicking units of enzyme for 60 min at 37°C (Fig. 5b). A complete loss of form I DNA and full-length single-stranded linear DNA occurred. The DNA from each reaction sedimented as a single peak. The calculated Mn values, 2.0×10^5 and 1.7×10^5, respectively correspond to 21 and 25.2 single-strand scissions per molecule. Therefore, since each DNA molecule contained an expected 25 dimers per molecule, this indicates that the phosphodiester bond in the DNA at the sites of pyrimidine dimers was cleaved before the enzyme produced cleavages in other DNA molecules.

This hypothesis predicts that completely incised strands will appear as the fraction of DNA as form I decreases, while only minor amounts of intermediate species would be detected as the reaction proceeds. Analysis of the time course of accumulation of single-strand breaks by action of the endonuclease V (137.5 nicking units) on DNA containing an average of 25 dimers per molecule showed this was the case (Fig. 6). Analysis of the DNA by sedimentation in alkali revealed an accumulation of low molecular weight DNA with a peak centered in the position of fully incised DNA (Fig. 5b). This accumulation of fragmented DNA does not evidently occur by a gradual stepwise shifting of the mass of DNA from a high molecular weight to lower molecular weights.

DISCUSSION

Our analysis of the action of endonuclease V preparations on a population of UV-irradiated ColE1 DNA molecules has revealed that the rate of loss of form I DNA and accumulation of form III DNA is clearly dependent on the average number of pyrimidine dimers in the original DNA substrate (Figs. 1 and 2). Further analyses of the distribution of strand scissions following limiting enzyme treatment of ColE1 DNA containing 25 dimers per molecule has revealed that most, if not all, pyrimidine dimers in a single DNA molecule are sites of cleavage by the nicking activity before similar scissions are produced in other DNA molecules. This result provides a reasonable explanation for the greater than expected production of form III DNA early in the incision reaction. Assuming cleavage at all pyrimidine dimers, comparison of the expected versus observed double-strand break frequency in DNA molecules containing various numbers of dimers per molecule revealed less than 20% differences.

We propose a processive model for the action of the T4 endonuclease V in which the enzyme produces the glycosylic bond scission with subsequent strand

scission at the site of the first dimer in the substrate DNA and then linearly diffuses along that DNA to encounter additional dimers. This process would continue until the sites of all dimers were cleaved in the DNA molecule on which the initial enzymatic action took place. The enzyme would eventually diffuse to other DNA molecules and the sequential process would be repeated.

If the endonuclease V diffuses on and off a DNA molecule, it is only necessary to suggest that the nearest dimer to the enzyme would be located on the same DNA molecule to explain the apparent processivity. To test this possibility, the following estimation was made of the distance between pyrimidine dimers within a ColE1 DNA molecule versus the distance between pyrimidine dimers on separate DNA molecules for the conditions present at the start of the reaction: 1) The duplex length of a ColE1 DNA molecule (Na^+β-form) equals 2.1 μm so that the average distance between dimers in DNA containing 25 dimers per molecule should be 0.08 μm; 2) for a DNA concentration of 57 μg/ml there would be 8.14×10^{12} molecules/cm^3 or 1 DNA molecule/1.23×10^{-13} cm^3 so that the average distance between DNA molecules would be 0.49 μm. Thus a distance between ColE1 DNA molecules is only approximately 5 times greater than the intramolecular distance between dimers in a ColE1 molecule containing 25 dimers/molecule. This estimation would suggest that one out of every five DNA scissions might occur on a DNA molecule other than the one on which the previous scission had been made if the enzyme diffused freely. However, at this DNA concentration, incision at all 25 dimers in some DNA molecules was demonstrated to occur before other DNA molecules were nicked (Figs. 5, 6). This suggests that the processive mechanism of endonuclease V is not simply due to the proximity of the nearest available substrate. It is of interest to point out that the restriction enzyme EcoRI (and probably any restriction enzyme which produces partial DNA fragments under conditions of limiting enzyme) does not act in a processive manner on limiting concentrations of plasmid DNA containing more than one EcoRI site (21). Since both EcoRI and T4 endonuclease V act at a limited number of discrete sites on DNA, there must be a fundamental difference between the modes of interaction of these enzymes with DNA containing multiple substrate sites.

If the enzyme does diffuse along and remain associated with the DNA, the forces which keep the enzyme and DNA together must be relatively weak since Seawell et al. (13) have demonstrated that T4 endonuclease V has no strong binding affinity for unirradiated DNA. This was demonstrated by showing that a vast excess of unirradiated DNA does not competitively inhibit binding of endonuclease V to irradiated DNA as measured by a filter binding assay.

Although the endonuclease V from T4-infected E. coli had previously been reported to specifically incise strands of DNA on the 5' phosphate sides of the pyrimidine dimers, recent studies have indicated a more complex, two-step reaction (4-6). It appears that the first step involves a 5' N-glycosylic cleavage of the dimerized pyrimidine. In a second step, the phosphodiester bond between the two pyrimidines comprising the dimer is cleaved. The question has not been resolved whether these two activities reside in one protein molecule, or whether the T4 denV gene codes for a protein with only glycosylase activity. The processive model predicts various characteristics of an endonuclease V-treated DNA population. For example, if the glycosylic bond scissions and the single-strand scissions were mediated by separate enzymes and the glycosylase were in vast excess, many more glycosylic breaks should be made compared to direct scissions. Comparison of the percent of form I DNA as determined by agarose gel electrophoresis versus alkaline velocity sedimentation have revealed no significant difference in the relative proportion of molecules containing only alkali labile sites to those containing single-strand breaks (Figs. 5, 6). This would suggest that the two activities are intrinsically coupled or reside in one protein molecule, or that the apurinic-apyrimidimic endonuclease is in vast excess. It might be argued that the glycosylase activity is the processive enzyme while a separate apurinic-apyrimidimic enzyme is only apparently processive because the glycosylase-nicked substrate has been produced in a processive manner. If the apurinic-apyrimidimic enzyme were in excess or its activity were closely coupled in one or two proteins, most or all glycoslyic breaks would be converted to single-strand scissions, thus accounting for the production of double-strand scissions by a simple accumulation of multiple single-strand scissions in individual DNA molecules. This we consider the most likely explanation of our observations.

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