RELATIONSHIP BETWEEN S1 ENDONUCLEASE-SENSITIVITY AND NUMBER OF SUPERHELICAL TURNS IN A NEGATIVELY-TWISTED DNA

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1. Introduction

A deficiency of helical turns in a covalently closed, circular DNA causes the whole molecule to twist into a compact negative supercoil. This negative superhelicity introduces localized unwinding of helical base pairs into the molecule. S1, a single strand-specific endonuclease from *Aspergillus oryzae* [1] can cleave both strands of the twisted DNA at the unpaired regions to generate unit length linear duplex molecules with intact single strands [2–5]. The reaction proceeds by two steps;

(1) Cleavage occurs in either one of two strands;
(2) The nicked, circular DNA is cleaved on the opposite strand at or near the nicks to yield a linear molecule.

However, it is still unknown what number of superhelical turns is necessary to produce the unpaired regions, sensitive to S1 endonuclease cleavage, into the DNA. To clear this, a set of closed, circular DNA species differing by one turn in their number of superhelical turns which is generated by limited digestion with *Haemophilus gallinarum* DNA-relaxing enzyme [6], was further digested with S1. The resulting DNA sample was analyzed by agarose gel electrophoresis. The experimental results obtained with the DNAs of Colicin E1 and SV40 demonstrate that S1 cannot cleave DNA molecules containing only 40% and 30% of superhelical turns of native DNA, respectively.

2. Materials and methods

2.1. *DNAs*

Colicin E1 DNA was prepared from *E. coli* strain JC411 (col E1)thy$^-$ according to the combined procedure in [7,8]. SV40 DNA was prepared from purified virus particles of strain 777 [9]. The λ DNA was prepared from *E. coli* M65 (λ*c*I857s7) by the method in [10].

2.2. *Enzymes and reactions*

The *Haemophilus gallinarum* superhelical DNA-relaxing enzyme was prepared as in [6]. The reaction mixtures (20 μl) containing 20 mM Tris–HCl (pH 7.5), 5 mM $MgCl_2$, 10 mM NaCl, 0.4 μg superhelical DNA and the indicated amount of the relaxing enzyme were incubated at 37°C for 1 h. One unit of DNA-relaxing activity is defined as the amount of the enzyme that converts 0.4 μg superhelical DNA into a completely relaxed form under the above conditions. S1 endonuclease provided by Kaken Chem. Co. was further purified as in [5]. Sequential digestion of the relaxing enzyme-treated DNA with S1 was performed as follows: to 20 μl reaction mixture of DNA-relaxation, the indicated amount of S1 and sodium acetate buffer (pH 5.0), NaCl and $ZnSO_4$ were added in 30 μl total vol. to give final conc. 50 mM, 50 mM and 1 mM, respectively. The incubation was performed at 37°C for 30 min. At pH 5.0, the relaxing enzyme is not functional [6]. The S1 reaction was stopped by addition of 3.3 μl 1 M Tris–HCl (pH 8.5) and 7 μl 0.1 M EDTA. One unit of S1 activity is defined as the amount of the enzyme that converts 50% of 0.4 μg single-stranded λ DNA to the acid-soluble form under the above conditions. After the S1 digestion, 5 μl 0.05% bromphenol blue–90% glycerol were added and the resulting DNA sample was analyzed by agarose gel electrophoresis.

2.3. *Agarose gel electrophoresis*

Agarose tube gels (6 × 200 mm) and horizontal

slab gels (15 X 20 X 0.3–0.4 cm) were prepared and electrophoresed in Tris–acetate buffer (20 mM Tris–HCl, 10 mM sodium acetate, 10 mM NaCl and 1 mM EDTA, pH 7.5) at 37°C under the conditions in the figure legends. The DNA bands were stained with ethidium bromide (0.5 μg/ml) (purchased from Sigma Chem. Co.), visualized using short-wavelength ultraviolet light, and photographed as in [6].

3. Results and discussion

Fig.1 shows the cleavage patterns of colicin E1 DNA (a) and SV40 DNA (b) by S1 as a function of enzyme concentration. The efficiency of conversion of superhelical DNA to the unit length linear form was proportional to the amount of enzyme. With 5 units S1, the superhelical DNAs of colicin E1 and SV40 were converted completely to the unit length linear form (>85%) with slight production of the nicked form (~10%) and of DNA fragments smalller than the unit length (~5%).

Fig.2 shows the agarose gel electrophoretic patterns of superhelical Colicin E1 DNA (a) and SV40 DNA (b) as a function of *H. gallinarum* DNA-relaxing enzyme concentration. In the gels, the electrophoretic mobility was a function of the number of superhelical turns. The relaxation proceeded proportionally according to the amount of enzyme. With sufficient enzyme, almost all the DNA molecules of both DNAs were converted into a completely relaxed form (track 5). Whereas, with a small amount of the enzyme, these DNAs were distributed into a set of covalently closed, circular DNA species differing by one turn in their number of superhelical turns ranging from the original superhelical DNA to the completely relaxed DNA (including the nicked DNA).

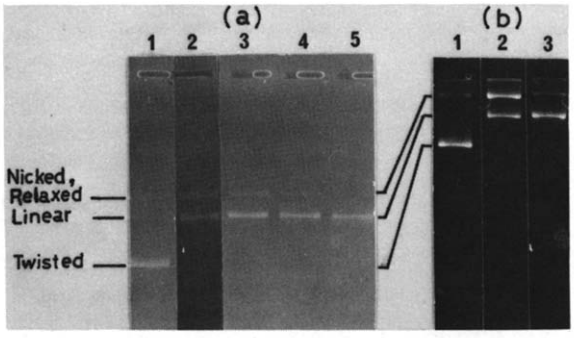

Fig.1. Conversion of superhelical colicin E1 and SV40 DNAs to the unit length linear form by S1-endonuclease. The 0.4 μg superhelical DNA (dissolved in 20 μl 20 mM Tris–HCl (pH 7.5), 5 mM $MgCl_2$ and 10 mM NaCl) was digested with the following amounts S1 as described in section 2. The digests (25 μl aliquots, to avoid overflow from the wells into which the DNA samples were to be applied) were electrophoresed in horizontal slab gels as in section 2. (a) Colicin E1 DNA, in 0.9% agarose at 4 V/cm for 3 h: track 1, without S1; track 2, 1.5 units; track 3, 3 units; track 4, 4 units, track 5, 5 units. (b) SV40 DNA, in 1.2% agarose at 2.5 V/cm for 1.5 h: track 1, without S1; track 2, 1.5 units; track 3, 5 units.

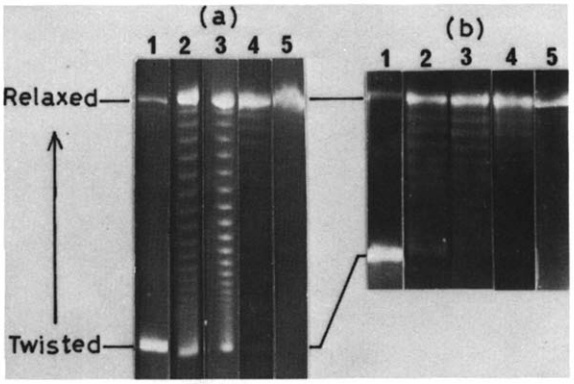

Fig.2. Agarose gel electrophoretic patterns of twisted DNA as a function of the amount of relaxing enzyme. The 0.4 μg twisted DNA was treated with the following amounts of relaxing enzyme as described in section 2. The DNA samples were electrophoresed as in section 2. (a) Colicin E1 DNA, in 0.9% agarose tube gels at 45 V for 15 h: track 1, without enzyme; track 2, 0.075 unit; track 3, 0.1 unit; track 4, 0.4 unit; track 5, 1 unit. (b) SV40 DNA, in 1.0% agarose tube gels at 100 V for 4 h: track 1, without enzyme; track 2, 0.125 unit; track 3, 0.25 unit; track 4, 0.5 unit; track 5, 1 unit.

To study the relationship between S1-sensitivity and the number of superhelical turns, all the members of the DNA species produced by the limited digestion with the relaxing enzyme were digested with 5 units of S1 under conditions where native superhelical DNA is completely digested. The resulting DNA sample was analyzed by gel electrophoresis. The data are shown in fig.3. In the experiment using colicin E1 DNA (a), the DNA species containing >12 superhelical turns disappeared completely to be converted into the linear and nicked forms, whereas those containing 0–11 turns remained (track 3). In track 3, an intense band just below the unit length linear DNA which overlapped the DNA species containing 5 turns was visible. It is due to a minor produc-

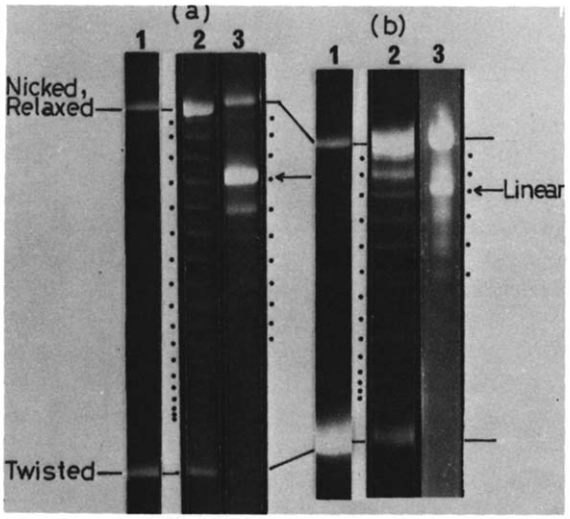

Fig.3. S1 endonuclease-cleavage patterns of partially relaxed DNA with the relaxing enzyme. The 0.4 μg twisted DNA was treated with 0.125 unit of relaxing enzyme as in section 2. The resulting DNA was digested with 5 units S1 and electrophoresed in agarose tube gels as in section 2. (a) Colicin E1 DNA, in 0.9% agarose at 45 V for 15 h: track 1, without both S1 and relaxing enzyme; track 2, with relaxing enzyme alone; track 3, with both relaxing enzyme and S1. (b) SV40 DNA, in 1.0% agarose at 35 V for 15 h: track 1, without both S1 and relaxing enzyme; track 2, with relaxing enzyme alone; track 3, with both relaxing enzyme and S1.

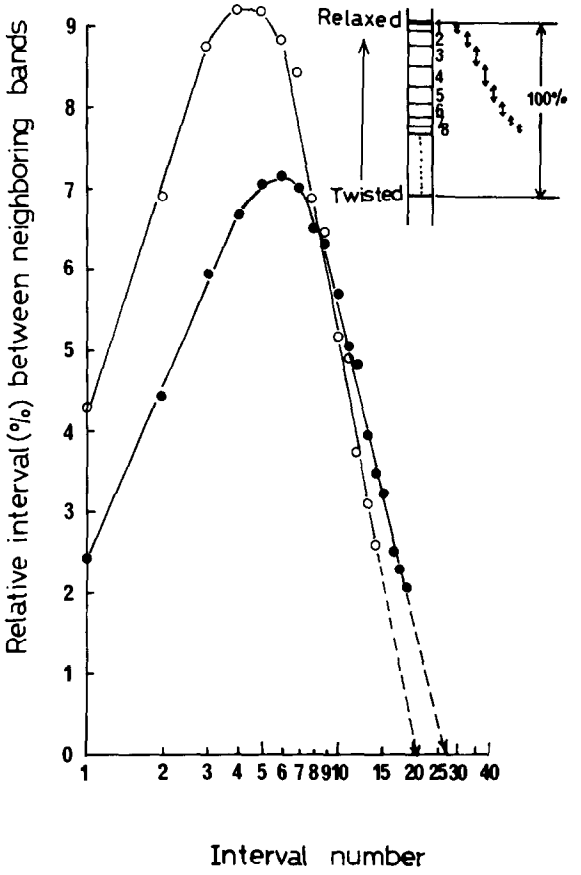

Fig.4. A plot of the relative interval (%) between neighboring bands against the interval number. The numbering of the intervals is shown in the inset. (●——●) Colicin E1 DNA; (○——○) SV40 DNA.

tion of a DNA fragment smaller by ~10% than the unit length, and produced by S1 digestion. In the case of SV40 DNA (b), the DNA species containing >7 turns were not seen, while those containing 0–6 turns were detectable (track 3).

The numbers of superhelical turns of native colicin E1 and SV40 DNAs were determined from each track 2 of fig.3 by the plotting of the relative interval (%) between neighboring bands against the interval number as in [11], as shown in fig.4. The total number of intervals (=no. superhelical turns) was estimated from the point of intersection of the extrapolated line and abscissa, where the interval converges to zero. Native colicin E1 and SV40 DNAs contain 27 and 20–21 superhelical turns, respectively (at 37°C in 20 mM Tris–HCl (pH 7.5), 5 mM $MgCl_2$ and 10 mM NaCl; almost the same conditions as that of the electrophoresis). The experimental results stated above demonstrate that 60% relaxed colicin E1 and 70% relaxed SV40 DNA molecules exhibit resistance to S1-cleavage.

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