IDENTIFICATION OF BLEOMYCIN-TREATED DNA FRAGMENTS BY MOLECULAR CLONING

Sir:

Bleomycin is a glycopeptide antibiotic with carcinostatic activity isolated from *Streptomyces verticillus*. It induces radical scission of DNA in the presence of ferrous ion and molecular oxygen by degradation of deoxyribose moiety[1,2]. Investigation of DNA damage by using 5'-end labeled short DNA sequence showed that bleomycin attacks G-C and G-T sites preferentially[3]. However, the exact pattern of degradation with a long stretch of DNA in nature is not known. Therefore, we have cloned bleomycin-treated fragments of sequence-determined plasmid DNA and sequenced them to see whether specific fragments are accumulated.

The dose effect of a component of bleomycin, bleomycin A_2, on degradation of a plasmid DNA, pAT153/*Pvu* II/8, is shown in Fig. 1. pAT153/*Pvu* II/8, a derivative of pAT153, is 3322 bp long and confers ampicillin-resistance to bacteria (G. G. BROWNLEE, personal communication). Bleomycin A_2 efficiently degrades this plasmid at above 3 μg/ml.

For cloning of the DNA fragments, the plasmid (10 μg) was incubated with 3 μg/ml of copper-free bleomycin A_2 (kindly supplied by Nippon Kayaku Co., Ltd.) and 0.1 mM $FeSO_4$ in 100 μl reaction mixture in the presence of 50 mM Tris-HCl, pH 8.0, and 10 mM mercaptoethanol for 30 minutes at 37°C. The reaction was stopped by addition of 10 μl 0.2 M EDTA and the DNA was isolated by a hydroxylapatite column (Elutip from Schleicher & Schuell, Inc.). The DNA fragments were then treated with 0.1 M piperidine for 15 minutes at 70°C, precipitated with ethanol and incubated with 2 units of calf intestine alkaline phosphatase (Boehringer) in a buffer containing 50 mM Tris-HCl, pH 9.0, and 1 mM spermidine for 30 minutes at 37°C. After heating at 70°C for 10 minutes and phenol-chloroform extraction the DNA was precipitated with ethanol, and incubated with T4-DNA poly-

Fig. 1. Dose effect of bleomycin A_2 on pAT153/*Pvu* II/8 DNA.

The plasmid DNA (2 μg) was incubated for 30 minutes at 37°C with 0.1 mM $FeSO_4$, 50 mM Tris-HCl, pH 8.0, and 10 mM mercaptoethanol (a), with 1 μg/ml (b), 3 μg/ml (c) or 15 μg/ml (d) of bleomycin A_2 and applied on 1.8% agarose gel.

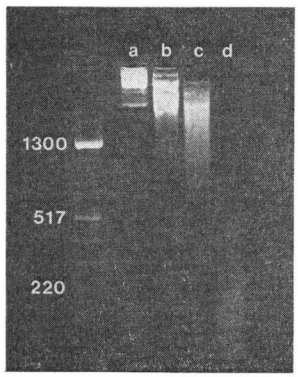
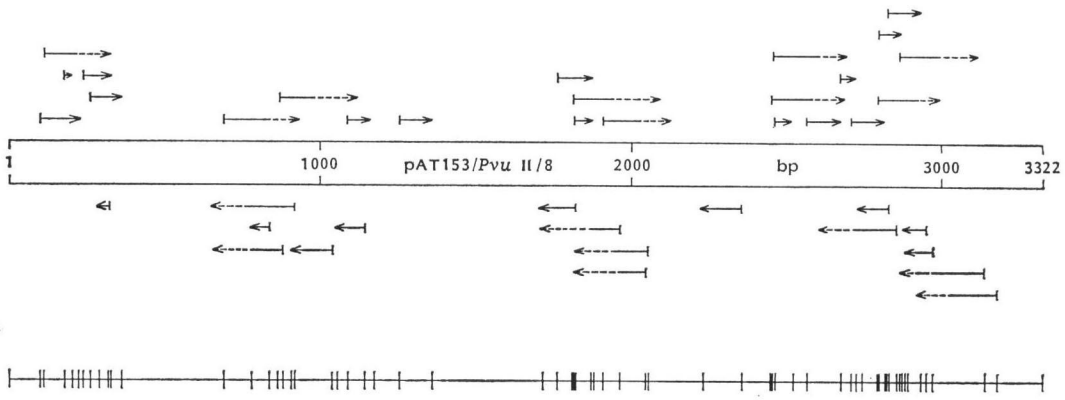


Fig. 2. Random scission of plasmid DNA by bleomycin A_2.

pAT153/*Pvu* II/8 DNA was fragmented by 3 μg/ml bleomycin A_2, and cloned and sequenced as described in the text. Reading of the sense strands is shown above and that of the complementary strand below. The position 1 indicates the *Eco* RI site in the plasmid. Vertical lines below indicate the sites of scission.



merase[4] (Takara Shuzo Co., Ltd.). After phenol-chloroform extraction and ethanol precipitation the bleomycin A_2-treated DNA fragments were ready for ligation to *Sma* I-cut M13mp9 vector. The ligated mixture was then added to competent *Escherichia coli* JM101 for transformation and recombinant plaques were selected[5]. The single stranded DNA was isolated and sequenced as described by SANGER *et al.*[6].

Because bleomycin A_2-treated DNA fragments may not have blunt ends and since they also possess carboxymethyl phosphate ester at the 3′-end[7], they were not ligated with the blunt end phage vector. We found that treatment of DNA fragments with piperidine, alkaline phosphatase and T4-DNA polymerase was effective for ligation and transformation.

As shown in Fig. 2, most cloned fragments were 100~200 bp long and the sites of scission were almost randomly dispersed throughout the sequence of pAT153/*Pvu* II/8. Breakage of cellular DNA probably accounts for the antitumor and antibacterial activity of bleomycin. Supercoiling or chromatin structure may influence susceptibility of DNA to bleomycin *in vivo*. But otherwise it is likely that bleomycin induces DNA scission almost randomly *in vivo*.

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