Vol. 117, No. 3, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
December 28, 1983 Pages 916-922

NUCLEOTIDE SEQUENCE CLEAVAGE OF GUANINE-MODIFIED DNA WITH AFLATOXIN B_1, DIMETHYL SULFATE, AND MITOMYCIN C BY BLEOMYCIN AND DEOXYRIBONUCLEASE I

Tadashi Suzuki, June Kuwahara, and Yukio Sugiura*

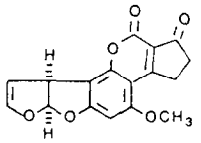
Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606, Japan

Received November 14, 1983

Summary : In guanine-modified DNA with aflatoxin B_1, dimethyl sulfate, and mitomycin C, the nucleotide sequence cleavage of bleomycin or DNase I has been investigated using ^{32}P-labeled DNA fragments. In contrast with cis-dichlorodiammine platinum(II) which induces intrastrand crosslinking of guanine N7 atoms, these mono-covalent modifiers did not give remarkable alteration of the DNA nucleotide cleavage mode by bleomycin and DNase I. From the viewpoint of combination drug, it is of interest that mitomycin C promoted the DNA cleavage activity of bleomycin.

Bleomycin cleaves isolated DNA preferentially at G-C(5'→3') and G-T (5'→3') sequences.[1-4] Several intercalating drugs induce the alteration of nucleotide sequence specificity in DNA cleavage by bleomycin.[5] In the presence of actinomycin D, the preferred sequence groups of bleomycin were shifted from G-C to G-A and G-T sequences. Distamycin A masked the cleavage at G-T and G-A sequences, and produced higher specificity for G-C sequences than that of bleomycin only. It is known that cis-dichlorodiammine platinum (II)(cis-DDP) forms intrastrand crosslinking of guanine N7 atoms in DNA.[6] cis-DDP also changes the sequence-specific cleavage of DNA by bleomycin, masking cutting sites near oligo(dG) sequences, and stimulating cutting elsewhere.[7] Herein, we address the related question of whether prior mono-covalent guanine binding of aflatoxin B_1, dimethyl sulfate, and mitomycin C to DNA might alter the specific nucleotide cleavage of bleomycin or endonuclease DNase I. The observed *in vitro* sequence specificity has revealed that all guanine residues in DNA are potential targets for modification by aflatoxin B_1[8] and dimethyl sulfate.[9] The mono-functional

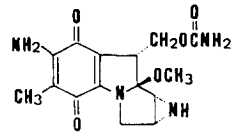
0006-291X/83 $1.50



Aflatoxin B_1

$(CH_3)_2SO_4$

Dimethyl Sulfate



Mitomycin C

binding of mitomycin C to DNA is highly guanine-specific,[10] and then mitomycin C-bleomycin drugs[11] as well as cis-DDP-bleomycin[12] have generally been used for combination chemotherapy of cancer treatment. From the viewpoint of the synergism in drug combination, therefore, it is also of interest to investigate the interaction of mitomycin C-modified DNA with bleomycin.

Experimental Sections

The restricted fragment of pBR 322 DNA digested by Hinf I was incubated with bacterial alkaline phosphatase, and the two 5'-termini were then rephosphorylated with $[\gamma\text{-}^{32}P]$ATP and polynucleotide kinase. After the labeling procedure, the DNA was digested with Hpa II or Hae III and the singly end-labeled 220- or 327-base pair fragment was isolated from 5 % polyacrylamide gels.

The aflatoxin B_1-modification was carried out by the addition of aflatoxin B_1(100 μM, in CH_2Cl_2) and chloroperoxybenzoic acid(500 μM, in CH_2Cl_2) to the appropriate end-labeled DNA preparation containing sonicated calf thymus DNA(1 μg) in 20 mM Tris-HCl buffer(pH 7.5). After the shaking at room temperature for 1 hr, the aqueous layer was extracted with chloroform and the DNA was recovered by cold ethanol precipitations. In the modification by dimethyl sulfate, the DNA sample in 20 mM Tris-HCl buffer(pH 7.5) was incubated with dimethyl sulfate(1 μl) at 20°C for 5 min. The mitomycin C-modification was performed by the mixing of mitomycin C(200 μM), sodium dithionite(10 μM), and the DNA in 20 mM Tris-HCl buffer(pH 7.5). After the incubation at 37°C for 1 hr, the DNA recovered by ethanol precipitations was dialyzed with a Millipore filter(VMWP 0.05 μM) for 40 min.

In the piperidine treatment, the modified DNA was degraded by heating at 90°C for 30 min in freshly prepared 1 M piperidine. The bleomycin cleavage of the DNA preparation was carried out by the addition of the bleomycin-iron complex(3.5 μM) and 2-mercaptoethanol(14 mM) in 20 mM Tris-HCl buffer(pH 8.3). After the incubation at 37°C for 6 min, EDTA was added to stop the reaction and then the DNA was recovered by cold ethanol precipitates. The DNase I cleavage of the DNA sample was initiated by the addition of

DNase I(Sigma) in 20 mM Tris-HCl buffer(pH 7.5) containing 9 mM $MgCl_2$ and 2 mM $CaCl_2$. After the reaction solution was incubated at 20°C for 3 min, the DNA was recovered by ethanol precipitates.

The sequence analytical electrophoresis was performed with 10 % polyacrylamide-7 M urea slab gels. The 220-base pair DNA fragment was used for the experiments in Figs. 1 and 2A, and the 327-base pair fragment in Figs. 2B, 3, and 4.

Results and Discussion

Figure 1 displays the gel electrophoretic results obtained with the 220-base pair DNA molecule for nucleotide sequence cleavages by piperidine after the treatment of aflatoxin B_1, dimethyl sulfate, and mitomycin C.

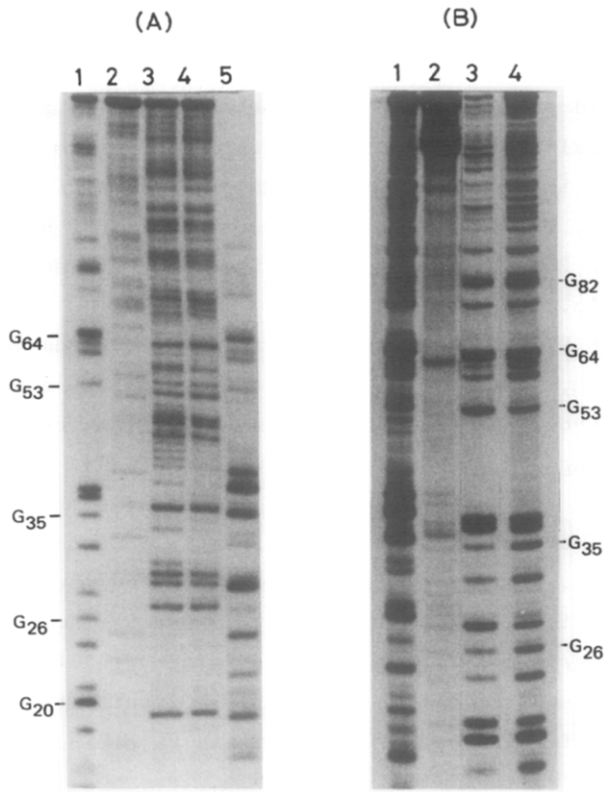


Figure 1 Nucleotide sequence cleavages by bleomycin for normal DNA and by piperidine for DNA after the treatment of aflatoxin B_1, dimethyl sulfate, and mitomycin C

Lanes 1-4 in (A) show the Maxam-Gilbert sequencing reactions for G, A>C, C+T, and C, respectively, and lane 5(also lane 1 of B) is control bleomycin digestion of DNA. Lanes 2, 3, and 4 in (B) are the products of piperidine treatment of DNA incubated with mitomycin C, aflatoxin B_1, and dimethyl sulfate, respectively. The G numbers on the figures represent length of the DNA fragments from the 5'-labeled terminus.

These reagents were used as point-modifier for guanine residues. As shown in the lanes 3 and 4 of (B), the alkali-labile sites induced by aflatoxin B_1 and dimethyl sulfate are clearly guanine bases. Indeed, Muench et al.[8] have also observed high specific modification of aflatoxin B_1 toward guanine residues in several DNA fragments from prokaryotic and eukaryotic sources. Model experiment clarified that the activated form of aflatoxin B_1 causes primarily covalent modification of guanine bases, and that its major stable reaction product with DNA is the guanine N7 adduct.[13] In Maxam-Gilbert method,[9] dimethyl sulfate-modified DNA is well-known to give guanine-specific cleavage by the heat-treatment. Mitomycin C reduced with $NaBH_4$ or $Na_2S_2O_4$ also appears to induce alkali-labile and fairly preferential cleavage at guanine residues. Recently, the O6 position of guanine was indicated as the major target of alkylation of $d(G_pC)$ by activated mitomycin C.[10] As shown in Figure 1A, bleomycin cleaved normal DNA fragments preferentially at guanine-pyrimidine(5'→3') sequences such as G-C and G-T. In the guanine-modified DNA obtained by the treatment of aflatoxin B_1 and dimethyl sulfate, bleomycin did not substantially alter the typical DNA cleavage mode(see Figure 2). Aflatoxin B_1 is bulky molecule. Nevertheless, it appears that the DNA duplex structural change induced by the point-modification of guanine residues with aflatoxin B_1 as well as dimethyl sulfate does not significantly affect on intercalative interaction between the bithiazole group of bleomycin and the guanine residues of DNA. In contrast, cis-DDP alters the bleomycin interaction with DNA and then the DNA conformational change induced by intrastrand crosslinking-modification with cis-DDP to guanine N7 residues obviously contributes to the pronounced alteration of the nucleotide sequence cleavage mode by bleomycin.[7] Actinomycin D which intercalates DNA preferentially at guanine rich sequences with binding site size of 3-5 base pairs, also evidently altered the nucleotide sequence-specific mode of DNA cleavage by bleomycin.[5]

Figure 3 shows the DNA cleavage pattern of mitomycin C-modified DNA by bleomycin. Although the alteration of bleomycin cleavage mode upon

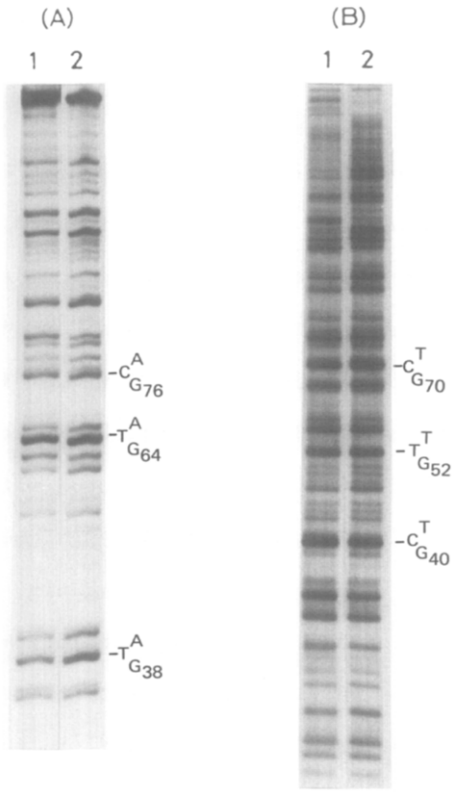


Figure 2 Sequence-specific cleavage of bleomycin for guanine-modified DNA with aflatoxin B_1 and dimethyl sulfate

Lanes 1 and 2 in (A) are the products of bleomycin treatment of normal DNA and DNA modified with aflatoxin B_1. Lanes 1 and 2 in (B) are the corresponding ones of normal DNA and DNA modified with dimethyl sulfate.

mitomycin C-binding DNA was remarkably small, mitomycin C stimulated the DNA cleavage activity of bleomycin. Indeed, it is known that a bleomycin-mitomycin C combination chemotherapy reveals synergism in the treatment of metastatic cervical cancer.[11]

When cis-DDP binds certain DNA fragments, on the other hand, exonuclease III activity stops at the point of G_n ($n>2$) sequences.[14] The observation is consistent with the proposal that cis-DDP creates an intrastrand link between adjacent guanine residues. Recently, endonuclease DNase I has also been shown capable of generating DNA cleavage inhibition pattern with actinomycin D.[15] The present DNA cutting of DNase I was not almost affected by the guanine-modification with aflatoxin B_1, dimethyl sulfate, and mitomycin C(see Figure 4).

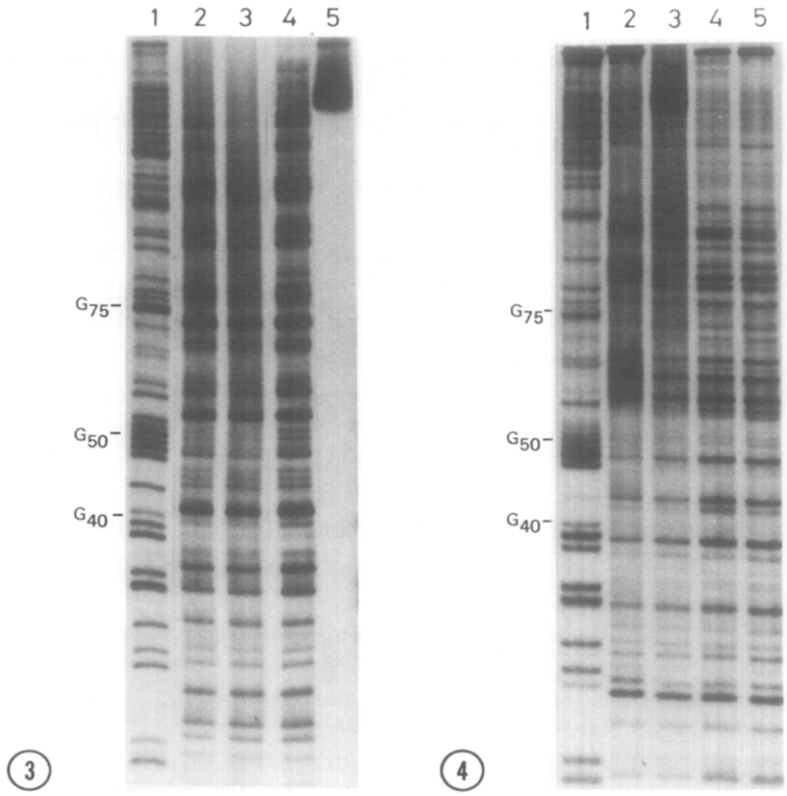


Figure 3 Sequence-specific cleavage of bleomycin for guanine-modified DNA with mitomycin C
Lanes 1, 4, and 5 show the standard G reaction, control bleomycin digestion of DNA, and control DNA incubated with mitomycin C, respectively. Lanes 2 and 3 are the products of bleomycin treatment of DNA incubated with mitomycin C at 37°C for 1 hr and 4 hr.

Figure 4 Nucleotide sequence cleavage of DNase I for guanine-modified DNA with aflatoxin B_1, dimethyl sulfate, and mitomycin C
Lanes 1 and 5 show piperidine treatment of DNA incubated with aflatoxin B_1 and control DNase I digestion of DNA. Lanes 2, 3, and 4 are the products of DNase I treatment of DNA incubated with aflatoxin B_1, mitomycin C, and dimethyl sulfate, respectively.

The present results clearly show the difference of DNA cleavage modes by bleomycin as well as by DNase I among the mono-covalent, crosslinked, and intercalated modifications of guanine residues. Binding of cis-DDP to DNA has a definite role in directing the cleavage mode of DNA by bleomycin, whereas bindings of aflatoxin B_1, dimethyl sulfate, and mitomycin C have little effect on the alteration of bleomycin cleavage mode. The differential responses of bleomycin and nuclease activities may have biological implication, because DNA in cells exist in different conformation relating to various gene functions.

References

1. D'Andrea,A.D. and Haseltine,W.A.(1978) Proc.Natl.Acad.Sci.USA 75, 3608-3612.

2. Takeshita,M., Grollman,A.P., Ohtsubo,E., and Ohtsubo,H.(1978) Proc.Natl. Acad.Sci.USA 75, 5983-5987.

3. Kross,J., Henner,W.D., Hecht,S.M., and Haseltine,W.A.(1982) Biochemistry 21, 4310-4318.

4. Sugiura,Y., Suzuki,T., Otsuka,M., Kobayashi,S., Ohno,M., Takita,T., and Umezawa,H.(1983) J.Biol.Chem. 258, 1328-1336.

5. Sugiura,Y. and Suzuki,T.(1982) J.Biol.Chem. 257, 10544-10546.

6. Lippard,S.J.(1982) Science 218, 1075-1081.

7. Maschrak,P.K., Sugiura,Y., Kuwahara,J., Suzuki,T., and Lippard,S.J.(1983) Proc.Natl.Acad.Sci.USA 80, in press.

8. Muench,K.F., Misra,R.P., and Humayun,M.Z.(1983) Proc.Natl.Acad.Sci.USA 80, 6-10.

9. Maxam,A.M. and Gilbert,W.(1980) Methods Enzymol. 65, 499-560.

10. Tomasz,M., Lipman,R., Snyder,J.K., and Nakanishi,K.(1983) J.Am.Chem.Soc. 105, 2059-2063.

11. Miyamoto,T.(1979) in Mitomycin C. Current Status and Developments, eds. Carter,S.K. and Crooke,S.T. (Academic Press, New York), pp. 163-171.

12. Perry,D.J., Weltz,M.D., Brown,Jr.,A.W., Henderson,R.L., Neglia,W.J., and Berenberg,J.L.(1982) Cancer 50, 2257-2260.

13. Büchi,G., Fowler,K.W., and Nadzan,A.M.(1982) J.Am.Chem.Soc. 104, 544-547.

14. Tullius,T.D. and Lippard,S.J.(1981) J.Am.Chem.Soc. 103, 4620-4622.

15. Lane,M.J., Dabrowiak,J.C., and Vournakis,J.N.(1983) Proc.Natl.Acad.Sci. USA 80, 3260-3264.