Vol. 183, No. 1, 1992 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1992 Pages 292-299

ENHANCEMENT AND ALTERATION OF BLEOMYCIN-CATALYZED SITE-SPECIFIC DNA CLEAVAGE BY DISTAMYCIN A AND SOME MINOR GROOVE BINDERS

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Received January 21, 1992

Summary: The effects of compounds which bind in the DNA minor groove of A•T rich sequences, on bleomycin-catalyzed site-specific DNA cleavage were investigated by a DNA sequencing technique. Distamycin A enhanced bleomycin-catalyzed DNA cleavage in G•C rich sequences such as 5'-GGGGC-3' (under scoring; the cleaved nucleotide). The cleavage in such a sequence in the presence of distamycin A was greater than that in the absence of distamycin A by as much as about 100 times. Neither Hoechst 33258, 4',6-diamidino-2-phenylindole (DAPI) nor berenil caused extensive enhancement. The results suggest that the distamycin-induced conformational changes of DNA through interactions other than the DNA minor groove binding in A•T-rich sequences are specifically suitable for the bleomycin action.

DNA is an important site of drug action, particularly for chemotherapy of neoplastic diseases. Appropriate modification of DNA conformation by DNA ligands is thought to enhance such drug action (1). Bleomycins are an important group of metal complexing glycopeptide antitumor agents differing in structure at a C-terminal cationic side chain (2, 3). Bleomycins recognize DNA molecule at the sequences of 5'-GC-3' or 5'-GT-3' by the bithiazole group (4, 5) and cause DNA scission at a deoxyribose moiety by the complexed Fe(II) interacting with O_2 (2). Differences in the terminal side chain of bleomycins have little effect on the recognition (6, 7). Pulmonary fibrosis is the common adverse effect of bleomycins (3). It was suggested that toxic O_2-derived species participate in the bleomycin lung toxicity (8) and that the susceptibility to the pulmonary fibrosis does not correlate with bleomycin-induced DNA damage (9). Therefore nontoxic

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0006-291X/92 $1.50

amplification of the DNA cleaving activity of bleomycin may enable more effective therapies with reduced doses of bleomycin and reduced pulmonary toxicity (1). It is very valuable to find DNA ligands which enhance bleomycin-catalyzed cleavage of DNA as a fundamental research for novel chemotherapies with bleomycins.

In this study, distamycin A (10-15), Hoechst 33258 (10,16-18), berenil (10,12,13) and 4',6-diamidino-2-phenylindole (DAPI) (1,10,17,19) were used as DNA ligands. These compounds have been shown to bind in the DNA minor groove in sequences of three or more consecutive A•T base pairs. We examined the effects of these compounds on the bleomycin-catalyzed site-specific cleavage of isolated DNA using a DNA sequencing technique.

Materials and Methods

Materials: Peplomycin, a kind of bleomycins, was obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan. 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI) was from Nacalai Tesque, Inc., Kyoto, Japan. Distamycin A hydrochloride and berenil (diminazene aceturate) were from Sigma. Hoechst 33258 was from Polyscience, Inc., Warrington, PE. [γ-^{32}P]ATP (6000 Ci/mmol) was purchased from Du Pont/NEN Research Products, Boston, MA.

Analysis of DNA Cleavage: DNA fragments were prepared from plasmid pbcNI which carries a 6.6-kilobase BamHI chromosomal DNA segment containing human c-Ha-ras-1 protooncogene (20). Singly labeled 261-base pair fragment (AvaI* 1645–XbaI 1905), 98-base pair fragment (AvaI* 2247–PstI 2344), and 337-base pair fragment (PstI 2345–AvaI* 2681) were obtained according to the method described previously (21). The asterisk indicates ^{32}P-labeling and nucleotide numbering starts with the BamHI site (20).

The standard reaction mixture in a microtube (Eppendorf) contained sonicated calf thymus DNA (50 μM nucleotide), [^{32}P]DNA fragment, and DNA ligand in 200 μl of 10 mM sodium phosphate buffer (pH 7.9). After incubation of the mixture at 37 °C for 5 min, 0.5 μM peplomycin and 0.5 μM $Fe^{II}SO_4(NH_4)_2SO_4$ were added and the mixture was incubated again at 37 °C for 5 min and electrophoresed with a 18 × 50 cm gel using a DNA sequencing system (LKB 2010 Macrophor) as previously described (22). Autoradiograms were obtained by exposure of x-ray film to the gel and scanned with a laser densitometer (LKB 2222 UltroScan XL) for the measurement of the relative amounts of oligonucleotides from treated DNA fragments. The preferred cleavage sites were determined by direct comparison of the positions of the oligonucleotides with those produced by the chemical reactions of the Maxam-Gilbert procedure (23).

Results

Fig. 1 shows effects of distamycin A, Hoechst 33258, DAPI, and berenil on bleomycin-catalyzed DNA cleavage. Distamycin A enhanced the cleavage in a site-specific manner and the maximal enhancing effect was observed at the concentration of 50 μM (Distamycin A / nucleotide = 1). Distamycin A of the concentration 10 ~ 200 μM (Distamycin A / nucleotide = 0.2 ~ 4) enhanced the cleavage and an excess of distamycin A (500 μM) protected DNA from the

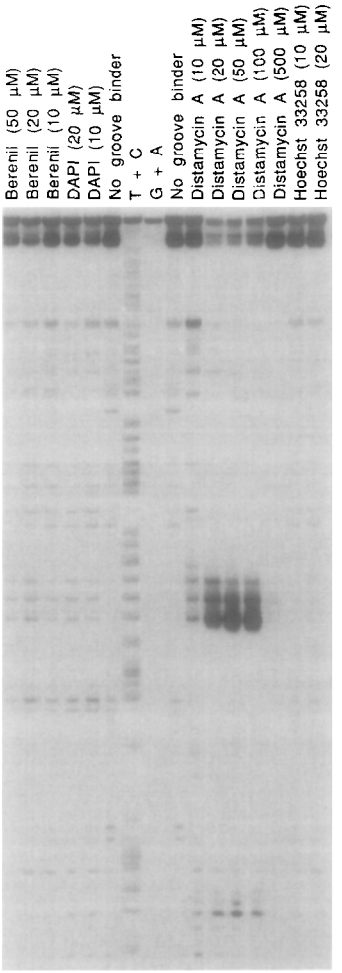

Fig. 1. Site specificity of DNA cleavage induced by peplomycin in the presence of distamycin A, Hoechst 33258, Berenil, or DAPI.

(A) The ^{32}P 5' end-labeled 337-base pair fragment (PstI 2345–AvaI* 2681) in 200 μl of 10 mM sodium phosphate buffer at pH 7.9 containing sonicated calf thymus DNA (50 μM nucleotide) was incubated at 37 °C for 5 min in the presence or absence of DNA binding compounds. Then 0.5 μM peplomycin and 0.5 μM $Fe^{II}SO_4(NH_4)_2SO_4$ were added and the mixture was incubated at 37 °C for 5 min. The treated DNA fragments were electrophoresed on an 8% polyacrylamide, 8 M urea gel (18 × 50 cm) using an DNA-sequencing system and the autoradiogram was obtained by exposing x-ray film to the gel. The lanes G + A, and T + C represent the patterns obtained for the same fragment after cleavage by the chemical methods of Maxam and Gilbert (23).

cleavage. Hoechst 33258, berenil and DAPI of the concentration about 20 μM (compound / nucleotide = 0.4) modulated the DNA cleavage moderately and protected DNA from the cleavage when they were added in excess. None of these compounds caused DNA cleavage without bleomycin.

The autoradiograms with DNA ligands of the concentrations showing their maximal effects were scanned with a laser densitometer (Fig. 2-4). In the

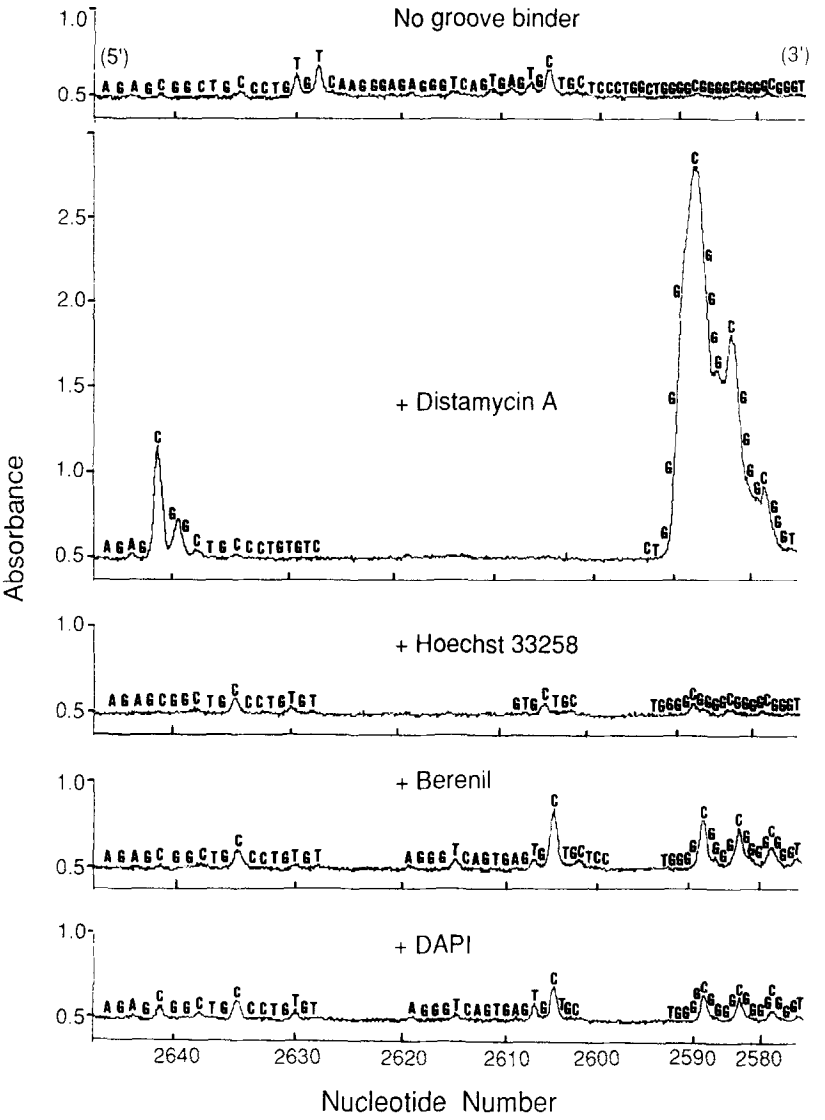

Fig. 2. Alteration of site specificity of peplomycin-induced cleavage of the 337-base pair DNA fragment by distamycin A, Hoechst 33258, Berenil, or DAPI.

The autoradiogram in Fig. 1 was scanned with a laser densitometer (LKB 2222 UltroScan XL). The horizontal axis, the nucleotide number of human c-Ha-ras-1 protooncogene starting with the BamHI site (20). Distamycin A, 50 μM; Hoechst 33258, 20 μM; berenil, 20 μM; DAPI, 20 μM.

absence of DNA ligand, bleomycin cleaved DNA at the sites of 5'-GC-3' and 5'-GT-3' sequences most extensively. The site specificity is in agreement with that previously reported (4,5). Distamycin A extensively enhanced the cleavage in the sequences (5'→3') of CTGGCTGGGGCGGGGCGGGG (2598-2579, Fig. 2), TGGGGCGGGGCGGGGCGGGT (2593-2574, Fig. 2), TGGTGGTGGGCGCCGGCGGT (1689-1708, Fig. 3),

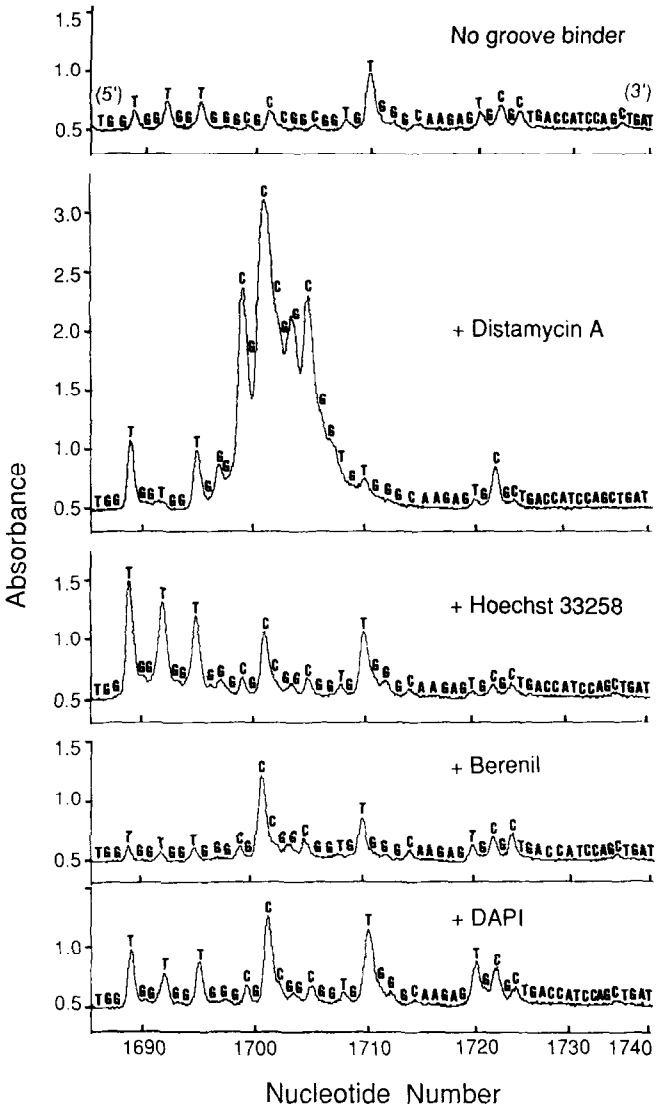

Fig. 3. Alteration of site specificity of peplomycin-induced cleavage of the 261-base pair DNA fragment by distamycin A, Hoechst 33258, Berenil, or DAPI.

Analysis with the ^{32}P 5' end-labeled 261-base pair fragment (AvaI* 1645–XbaI 1905) was performed as described in Figs. 1 and 2. Distamycin A, 50 μM; Hoechst 33258, 20 μM; berenil, 20 μM; DAPI, 20 μM.

GTGGTGGGCGCCGGCGGTGT (1691-1710, Fig. 3), TGGGCGCCGGCGGTGTGGGC (1695-1714, Fig. 3), and ACAGGTGGGGCCAGGCCGGC (2264-2283, Fig. 4) (underscoring; the cleaved nucleotide), while it inclined to protect 5'-GT-3' sequences from the cleavage. The other DNA ligands did not enhanced the cleavage so extensively. Hoechst 33258 and DAPI enhanced the cleavage only moderately in some of 5'-GGT-3' and

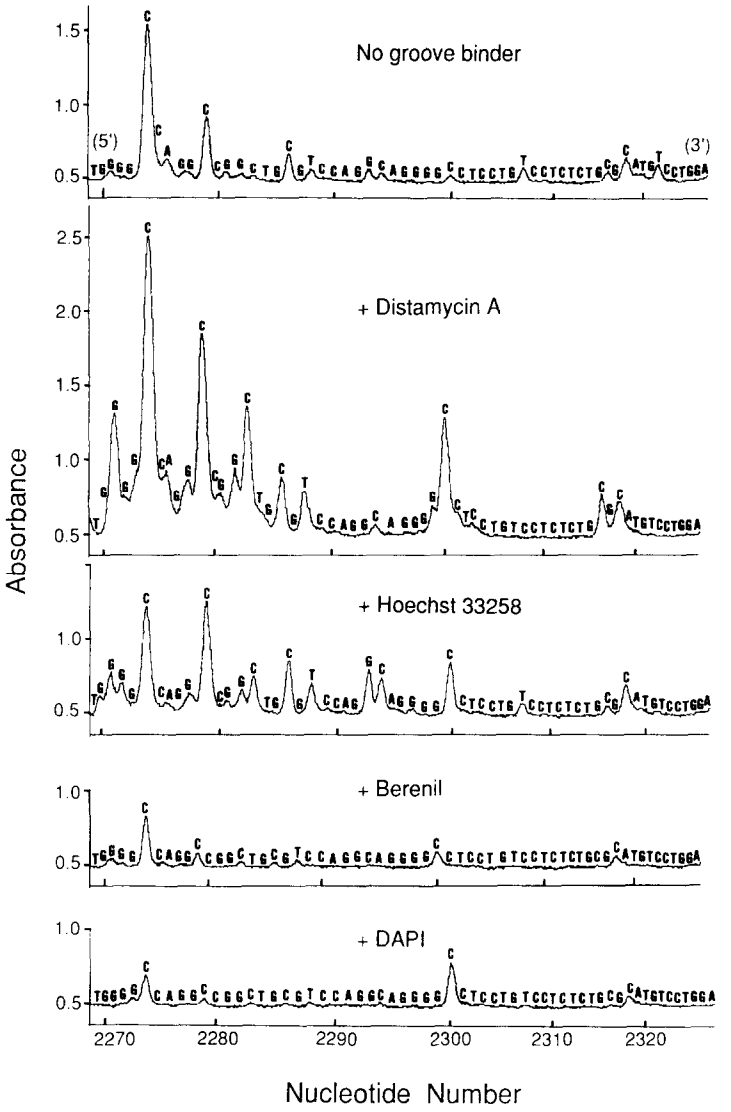

Fig. 4. Alteration of site specificity of peplomycin-induced cleavage of the 98-base pair DNA fragment by distamycin A, Hoechst 33258, Berenil, or DAPI.

Analysis with the ^{32}P 5' end-labeled 98-base pair fragment (AvaI* 2247–PstI 2344) was performed as described in Figs. 1 and 2.

Distamycin A, 50 μM; Hoechst 33258, 20 μM; berenil, 20 μM; DAPI, 20 μM.

5'-GGC-3' sequences. Berenil enhanced the cleavage only moderately in some of 5'-GC-3' sequences.

Discussion

Among the DNA groove binders used, distamycin A was the most effective in enhancing the bleomycin-catalyzed DNA cleavage. The extensive enhancement of

the cleavage was observed in G•C-rich sequences such as 3'-GGGGC-5'. The cleavage in such a sequence in the presence of distamycin A was greater than that in the absence of distamycin A by as much as about 100 times (Fig. 2). The present result suggests that very extensive enhancement of the cleavage by distamycin A is preferentially induced in consecutive G•C base pairs with a large size. Sugiura et al. reported that distamycin A inhibited the bleomycin-catalyzed DNA cleavages at 5'-GT-3' and 5'-GA-3' sequences and produced higher specificity for 5'-GC-3' sequences by about 3 times maximally (24). In their report, the data were obtained from analyzing a DNA sequence about 100 base pair and the sequence did not contain the consecutive G•C base pairs such as 3'-GGGGC-5' which are shown to be appropriate for the distamycin-induced extensive enhancement in the present study. It has been reported that distamycin A binds preferentially to A•T-rich stretches of 4-5 base pairs in the minor groove allowing many productive van der Waals and hydrogen bonding interactions and that an unusual conformation of DNA with a high positive propeller twist is induced (10-14). Recently Churchill et al. reported that distamycin A also occupies a site of a 5'-ACTA-3' sequence with high affinity using hydroxyl radical footprinting (15). G•C-rich sequences are weak binding sites for distamycin A (10). From the view point of sequence specificity of DNA binding by distamycin A, it is interesting that distamycin A enhance the bleomycin-catalyzed DNA cleavage in G•C-rich sequences without flanking A•T-rich sequences. The sites of local distortion or unwinding in DNA are reported to be particularly favorable for bleomycin to bind to and cleave (25,26). In hydroxyl radical footprinting experiments with the DNA fragments used, distamycin A bound to some sequences other than A•T-rich sequences (data not shown). Distamycin A may bind to the DNA fragments at or near the G•C-rich sequences in some way to change DNA conformation into a form suitable for bleomycin action. Other possibilities, however, are not excluded. For example, distamycin may recognize the DNA-bleomycin complex formed in the G•C rich sequences and bind to it to form a ternary DNA-bleomycin-distamycin complex, inducing enhancement of the DNA cleavage in the sequences.

Hoechst 33258, berenil, and DAPI bind to DNA preferentially in the minor groove of A•T-rich sequences as distamycin A does. However, extensive enhancement of the DNA cleavage was not observed with Hoechst 33258, berenil, and DAPI. The results shows that groove binders for A•T-rich sequences does not necessarily cause extensive enhancement. Hoechst 33258, berenil, and DAPI induced only moderate enhancement and protection. As a result of these two mutually competing effects, the bleomycin-catalyzed DNA cleavage was slightly enhanced by the compounds.

Enhancement of DNA cleaving activity of bleomycin may improve the therapies with bleomycin since the adverse effect may not due to DNA cleaving activity (9). Utilization of DNA ligands may lead to more effective drug usage in chemotherapy of neoplastic diseases (1). Further research is necessary to elucidate DNA conformational changes by DNA ligands and resulting enhancement of drug action.

Acknowledgments

We are grateful to Professor Masayuki Ikeda for his encouragement throughout this work. This work was supported in part by a research grant from the Fujiwara Foundation of Kyoto University, Grants-in-Aid for Scientific Research No 03258219 from the Ministry of Education, Science and Culture of Japan, and the Japan Society for the Promotion of Science for Japanese Junior Scientists.

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