Concerted DNA Recognition and Novel Site-Specific Alkylation by Duocarmycin A with Distamycin A†

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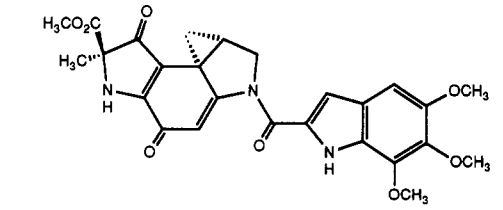
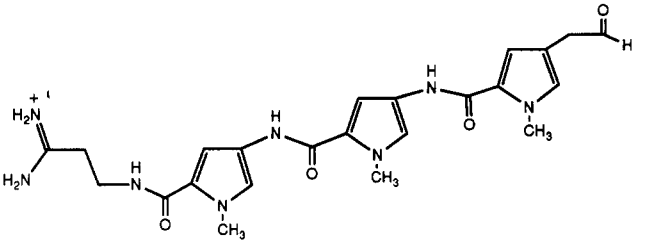
ABSTRACT: Duocarmycin A, a novel antitumor antibiotic, has a reactive cyclopropane ring, which has been reported to alkylate adenine at the 3′ end of sequences of three or more consecutive A or T in DNA [Boger, D. L., et al. (1990) *J. Am. Chem. Soc. 112*, 8961–8971]. In order to study the DNA recognition, the reaction of DNA with duocarmycin A was performed in the presence of DNA ligands. Distamycin A, berenil, Hoechst 33258, and 4′,6-diamidino-2-phenylindole (DAPI), which are minor-groove binders with affinity to A·T-rich sequences, were used. DNA-sequencing experiments showed that treatment of DNA with duocarmycin A plus distamycin A caused alkylation of guanine residues in G·C-rich sequences, which are not alkylated by duocarmycin A alone. Guanine alkylation by duocarmycin A was not observed with berenil, Hoechst 33258, or DAPI. HPLC product analysis showed that duocarmycin A reacted with a double-helical DNA octamer $d(CCCCGGGG)_2$ in the presence of distamycin A to produce duocarmycin A–guanine adduct, while duocarmycin A alone did not react with the octamer. Chromomycin A_3, which binds as a Mg(II)-coordinated dimer to G·C-rich sequences in the minor groove, inhibited the guanine alkylation by duocarmycin A in the presence of distamycin A. A footprinting experiment showed that there is a distamycin A-binding site close to the alkylated guanine residue. These results suggest that two different molecules, duocarmycin A and distamycin A, cooperatively recognize DNA sequences including consecutive G·C base pairs resulting in alkylation at the novel guanine sites. The cooperative drug recognition can be designated as "concerted DNA recognition".

Duocarmycin A is a member of a new class of antitumor antibiotics and contains a reactive cyclopropane ring as shown in Figure 1 (Takahashi et al., 1988; Yasuzawa et al., 1988; Gomi et al., 1992). It has been reported that the cyclopropyl moiety alkylates the N3 of adenine at the 3′ end of sequences of three or more consecutive A or T in double-stranded DNA (Boger et al., 1990a,b, 1991; Sugiyama et al., 1990). The recognition of the A·T-rich sequences by duocarmycin A is thought to be through binding to the minor groove of the sequences. Duocarmycin A is structurally similar to CC-1065, a previously discovered antitumor antibiotic, which binds in the minor groove of A·T-rich sequences and alkylates the N3 of adenine (Hurley et al., 1988; Boger et al., 1990b). The cellular target for CC-1065 is double-stranded DNA, and affinity for single-stranded DNA, RNA, or protein is small (Reynolds et al., 1986). In the case of duocarmycin A, double-stranded DNA is thought to be the cellular target.

The conformation of the DNA molecule in vivo is apparently different from that of DNA in simple buffered solutions. Therefore, it is very interesting to clarify whether alteration of the DNA conformation by DNA ligands affects the site specificity of duocarmycin A-induced DNA alkylation. The effects of DNA ligands are also of interest from the view point of chemotherapy of neoplasms because enhancement of antitumor drugs can lead to new multidrug chemotherapy. Distamycin A (Zimmer & Wähnert, 1986; Coll et al., 1987; Portugal & Waring, 1987; Neidle et al., 1987; Pelton & Wemmer, 1988; Churchill et al., 1990), berenil (Zimmer & Wähnert, 1986; Portugal & Waring, 1987; Neidle et al., 1987), Hoechst 33258 (Zimmer & Wähnert, 1986; Teng et al., 1988; Portugal & Waring, 1988; Parkinson et al., 1990), and DAPI[1] (Zimmer & Wähnert, 1986; Portugal & Waring, 1988; Wilson et al., 1990a,b) were used as DNA ligands. These DNA ligands have affinity to the relatively narrow minor groove of A·T-rich sequences, though there are some other binding sites. We examined the effects of these DNA ligands on duocarmycin



Duocarmycin A



Distamycin A

FIGURE 1: Structures of duocarmycin A and distamycin A.

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[1] Abbreviation: DAPI, 4′,6-diamidino-2-phenylindole.

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A-induced DNA alkylation with high-resolution polyacrylamide gel electrophoresis and HPLC product analysis.

MATERIALS AND METHODS

Materials. [γ-^{32}P]ATP (6000 Ci/mmol) was purchased from Du Pont-New England Nuclear. Restriction enzymes (*Ava*I, *Xba*I, *Pst*I) and T_4 polynucleotide kinase were purchased from New England, Biolabs, Beverly, MA. DNase I from bovine pancreas, Distamycin A hydrochloride, and berenil (diminazene aceturate) were from Sigma Chemical Co., St. Louis, MO. Duocarmycin A was from Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan. DAPI was from Nacalai Tesque, Inc., Kyoto, Japan. Chromomycin A_3 was from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Hoechst 33258 was from Polyscience, Inc., Warrington, PA. Calf intestine alkaline phosphate (1000 units/mL) and snake venom phosphodiesterase (3 units/mL) were purchased from Boehringer Mannheim. Deoxyoctanucleotide d(CCCCGGGG) was synthesized by an ABI 381A DNA synthesizer (Applied Biosystems), and the concentration was determined by complete digestion with calf intestine alkaline phosphate and snake venom phosphodiesterase to 2′-deoxyguanosine and 2′-deoxycytidine.

Preparation of DNA Fragments. ^{32}P 5′-end-labeled DNA fragments were prepared from plasmid pbcNI which carries a 6.6-kilobase *Bam*HI chromosomal DNA segment containing human c-Ha-*ras*-1 protooncogene (Capon et al., 1983) according to the method described previously (Yamamoto et al., 1989). Singly labeled 261-base pair fragment (*Ava*I* 1645–*Xba*I 1905), 341-base pair fragment (*Xba*I 1906–*Ava*I* 2246), 98-base pair fragment (*Ava*I* 2247–*Pst*I 2344), and 337-base pair fragment (*Pst*I 2345–*Ava*I* 2681) were obtained. The asterisk indicates ^{32}P-labeling and nucleotide numbering starts with the *Bam*HI site (Capon et al., 1983).

Analysis of DNA Damage. 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, 5 μM duocarmycin A was added and the mixture was incubated again at 20 °C for 60 min. The samples were treated with heat and piperidine and electrophoresed with a 12 × 16 cm gel or with a 18 × 50 cm gel using a DNA-sequencing system (LKB 2010 Macrophor) as previously described (Kawanishi & Yamamoto, 1991; Yamamoto & Kawanishi, 1991). 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 were those produced by the chemical reactions of the Maxam–Gilbert procedure (Maxam & Gilbert, 1980).

DNase I Footprinting. Footprinting measurements was performed according to Low et al. (1984). The reaction mixture (total volume 50 μL) contained the ^{32}P 5′-end-labeled 98-base pair fragment and sonicated calf thymus DNA (50 μM nucleotide) in 10 mM Tris–HCl buffer (pH 7.0) containing 10 mM NaCl, 0.4 mM $MgCl_2$, and 0.4 mM $MnCl_2$. After addition of duocarmycin A and/or distamycin A where indicated, the sample was preincubated at 37 °C for 5 min and digested with 0.05 unit/mL DNase I for 10 min at 20 °C. After ethanol precipitation, a solution of formamide–electrophoresis dye was added. Samples was loaded onto the gel after heating at 90 °C for 1 min.

HPLC Product Analysis. The reaction mixture (total volume 100 μL) containing d(CCCCGGGG)$_2$ (1 mM nucleotide) and duocarmycin A (0.1 mM) in 50 mM sodium cacodylate buffer at pH 7.0 was incubated at 0 °C for 1 h in the presence or absence of distamycin A (0.1 mM). The reaction mixture was extracted with water-saturated ethyl acetate (100 μL) to remove unreacted duocarmycin A and then analyzed after heating at 90 °C for 5 min. HPLC analysis was carried out using the CCPE II HPLC system (Tosoh, Tokyo, Japan) equipped with a Cosmosil 5C18 column (4.6 × 150 mm; Nacalai Tesque). Elution was undertaken at a flow rate of 1.5 mL/min with a linear gradient of acetonitrile from 0 to 50% (0–30 min) in aqueous ammonium formate (50 mM). Detection was carried out at 254 nm.

RESULTS

Effects of Various Concentrations of DNA Ligands on Duocarmycin A-Induced Site-Specific DNA Alkylation. Alkylation of DNA by duocarmycin A was monitored by gel electrophoretic analysis of DNA fragments after the heat and piperidine treatment. The DNA alkylated with duocarmycin A is cleaved by heat and/or piperidine treatment at the sites of alkylated bases (Boger et al., 1990a,b, 1991; Sugiyama et al., 1990). The sequence specificity of the DNA alkylation sites was determined by direct comparison of the positions of the oligonucleotides with those produced by the chemical reactions of the Maxam–Gilbert procedures. Figures 2 and 3 show the effects of various concentrations of distamycin A, berenil, Hoechst 33258, and DAPI on duocarmycin A-induced DNA alkylation. The autoradiograms were scanned with a laser densitometer (Figures 4 and 5).

In the absence of DNA ligands, duocarmycin A alkylated DNA at 5′-ATAT*AA*GCTG-3′ (1682 and 1683 in 261-bp fragment; Figure 2, lane A, band VI; Figure 4A1) and 5′-CAAA*A*GACT-3′ (2203 in 341-bp fragment; Figure 4A2) (italics indicate the alkylated nucleotides). The result is in agreement with the reported sequence specificity obtained from using different DNA fragments (Boger et al., 1990a,b). Distamycin A altered the alkylation pattern completely. In the presence of distamycin A, the alkylation of the adenine residues at the 3′ end of sequences of three or more consecutive A or T was completely inhibited (Figures 2–5), and interestingly, other adenine and guanine residues were alkylated (Table I). The novel guanine alkylation in G·C-rich sequences by duocarmycin A in the presence of distamycin A is a totally new finding. The sequences of 5′-(A·T)G(G·C)T*GG*-3′ are favorable for the guanine alkylation.

Berenil inhibited the alkylation at 5′-ATAT*AA*GCTG-3′ (1682 and 1683 in 261-bp fragment; Figure 2, lanes C1–4, band VI; Figure 4C1) and 5′-CAAA*A*GACT-3′ (2203 in 341-bp fragment; Figure 4C2), but the inhibition was not so strong as that by distamycin A. With berenil, duocarmycin A alkylated other adenine residues (Table I), but not guanine residues. Although the alkylation pattern was changed, the preference for adenine alkylation was kept. Hoechst 33258 showed inhibitory effects on the alkylation by duocarmycin A, except that it induced alkylation at the sequence of 5′-AGGC*A*GGGG-3′ (2295 in 98-bp fragment; Figure 3, lanes D1–4, band I). The inhibitory effects of DAPI were similar to those of berenil (Figures 2 and 3), whereas the enhancing effects were smaller (Figure 2, lanes E1–4, band I).

DNase I Footprinting of the Distamycin A and Duocarmycin A. To study the binding of duocarmycin A plus distamycin A to DNA, DNase I footprinting was carried out

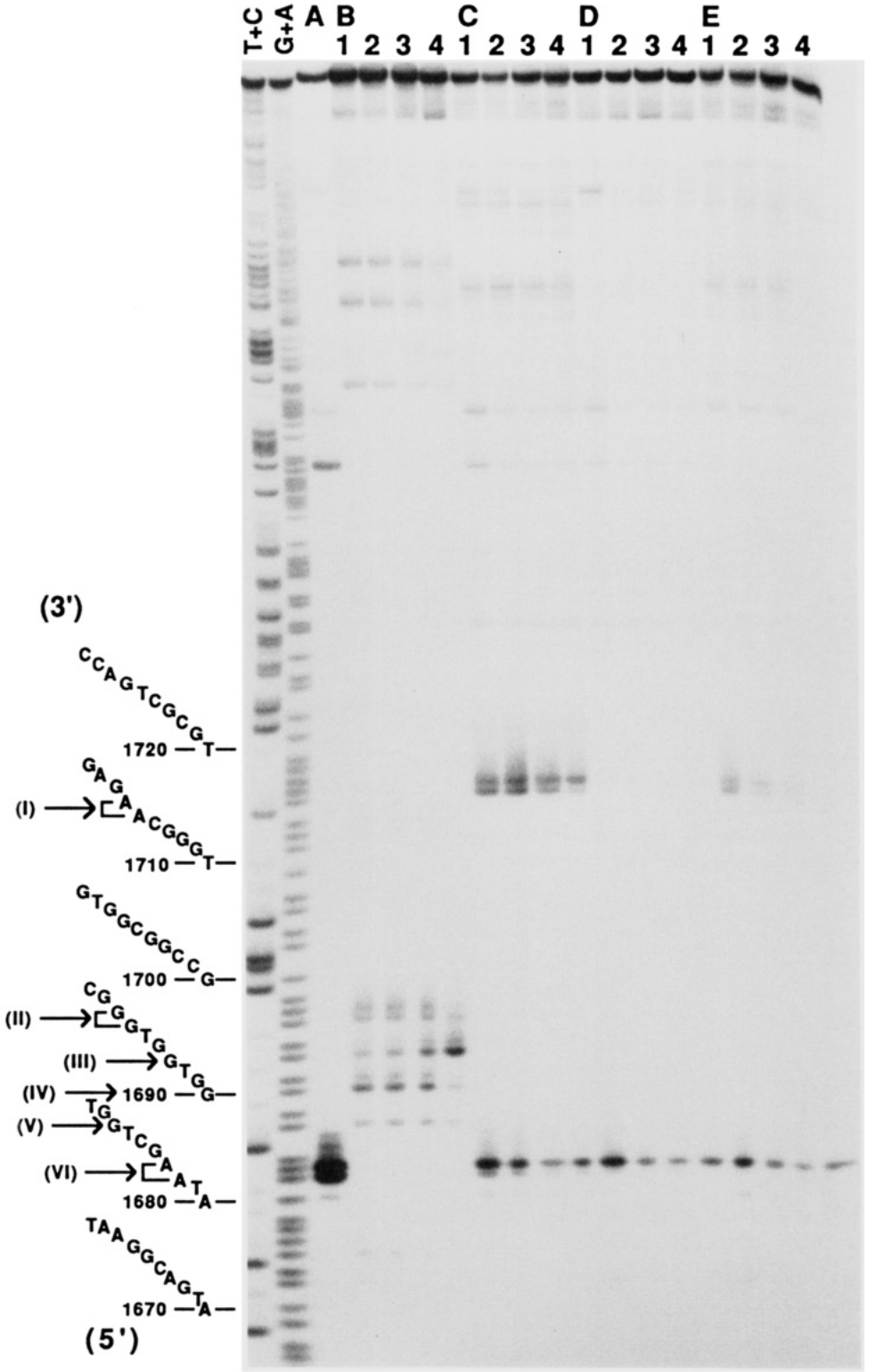

FIGURE 2: Autoradiogram of ^{32}P-labeled DNA fragments incubated with duocarmycin A in the presence of DNA ligands. The ^{32}P 5′-end-labeled 261-base pair fragment in 200 μL of 10 mM sodium phosphate buffer at pH 7.9 containing sonicated calf thymus DNA (50 μM nucleotide) was incubated at 37 °C for 5 min in the presence of indicated concentrations of DNA ligands. Then 5 μM duocarmycin A was added, and the mixture was incubated at 20 °C for 60 min. The treated DNA fragments were ethanol precipitated for removal of unbound drug, dissolved in 90 μL of 10 mM sodium phosphate buffer at pH 7.9, and heated at 90 °C for 5 min. After addition of 10 μL of piperidine, the DNA fragments were heated again at 90 °C for 20 min, precipitated with ethanol, dried under vacuum, and electrophoresed on an 8% polyacrylamide–8 M urea gel (18 × 50 cm) using an DNA-sequencing system. The autoradiogram was obtained by exposing X-ray film to the gel. The G + A and T + C lanes represent the patterns obtained for the same fragment after cleavage by the chemical methods of Maxam and Gilbert (1980). The nucleotide number of human c-Ha-*ras*-1 protooncogene starts with the *Bam*HI site (Capon et al., 1983). Key: (A) no ligand; (B) distamycin A; (C) berenil; (D) Hoechst 33258; (E) DAPI. Ligand concentration (μM): (1) 5; (2) 10; (3) 20; (4) 50.

using the ^{32}P 5′-end-labeled 98-base pair fragment which lacks sequences of three consecutive A or T. Duocarmycin A plus distamycin A causes guanine alkylation at the sequence of 5′-AGGT*G*GGGC-3′ (2270 in 98-bp fragment, Figure 3, lanes B1–4, band IV; Figure 5B2). Binding of distamycin A was detected at high concentration (50 μM) within the same sequence of 5′-AGGTGGGG-3′ (2266–2273 in 98-bp fragment; Figure 6, lane B3, arrow mark). The binding does not

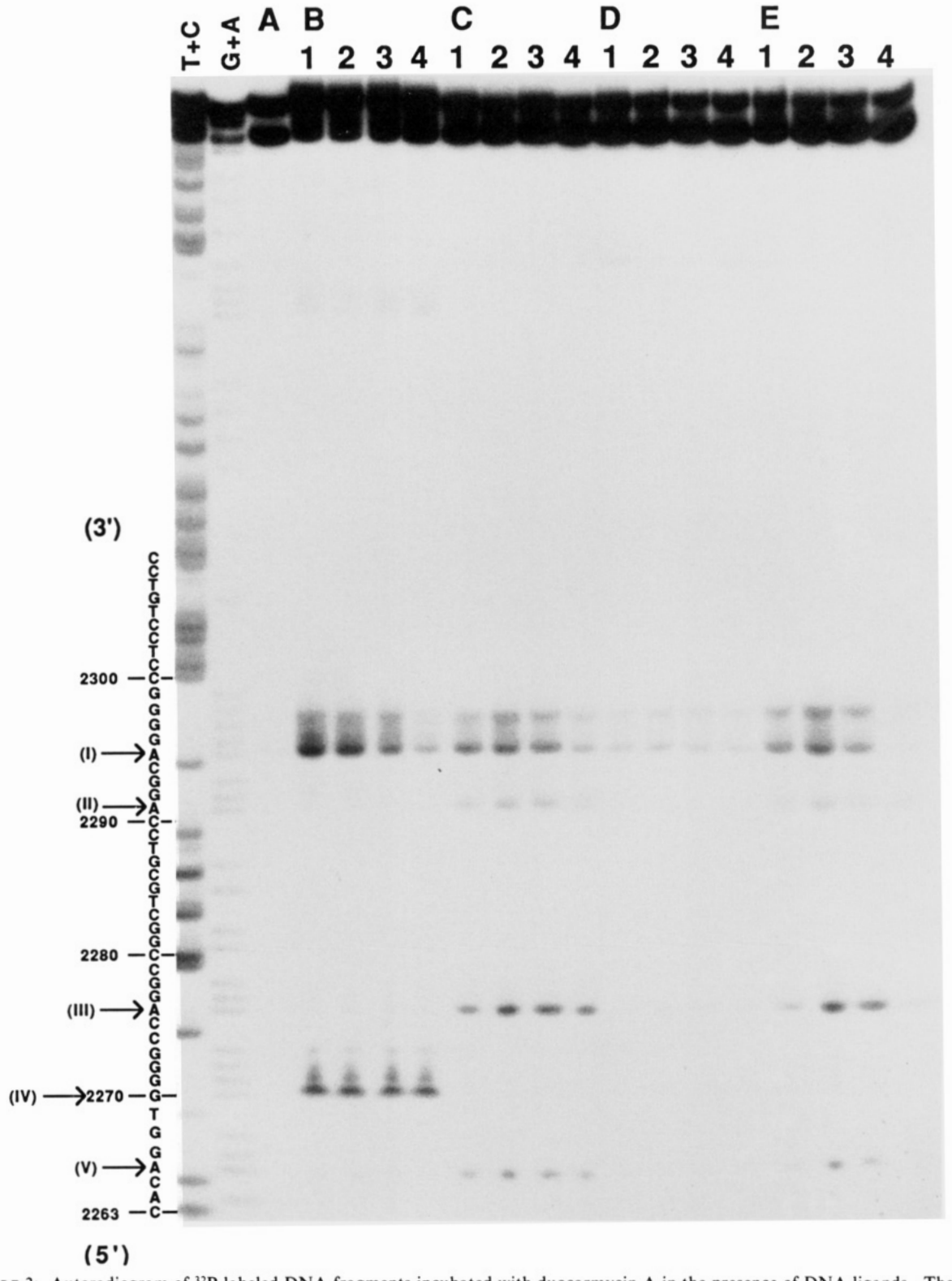

FIGURE 3: Autoradiogram of ^{32}P-labeled DNA fragments incubated with duocarmycin A in the presence of DNA ligands. The ^{32}P 5′-end-labeled 98-base pair fragment was treated with duocarmycin A in the presence of DNA ligands as described in Figure 1. Key: (A) no ligand; (B) distamycin A; (C) berenil; (D) Hoechst 33258; (E) DAPI. Ligand concentration (μM): (1) 5; (2) 10; (3) 20; (4) 50.

seem very strong because it was not detected with low concentrations of distamycin A (lanes B1 and B2). The footprint of duocarmycin A plus distamycin A was similar to that of distamycin A, although a band produced by duocarmycin A plus distamycin A-induced alkylation was observed additionally (Figure 6, lanes C and D, star). These results suggest that both duocarmycin A and distamycin A bind to the DNA within the sequence of only 8 base pairs (5′-AGGT*G*GGG-3′), causing guanine alkylation.

Inhibition of the DNA Alkylation by Chromomycin A_3 plus Mg(II). To investigate whether guanine alkylation by duocarmycin A in the presence of distamycin A occurs at the major-groove side or at the minor-groove side, the effect of chromomycin A_3 was examined using the ^{32}P 5′-end-labeled 98-base pair fragment. Guanine alkylation was inhibited by chromomycin A_3 in the presence of $MgCl_2$ (Figure 7). The inhibitory effect of chromomycin A_3 was not observed in the absence of $MgCl_2$.

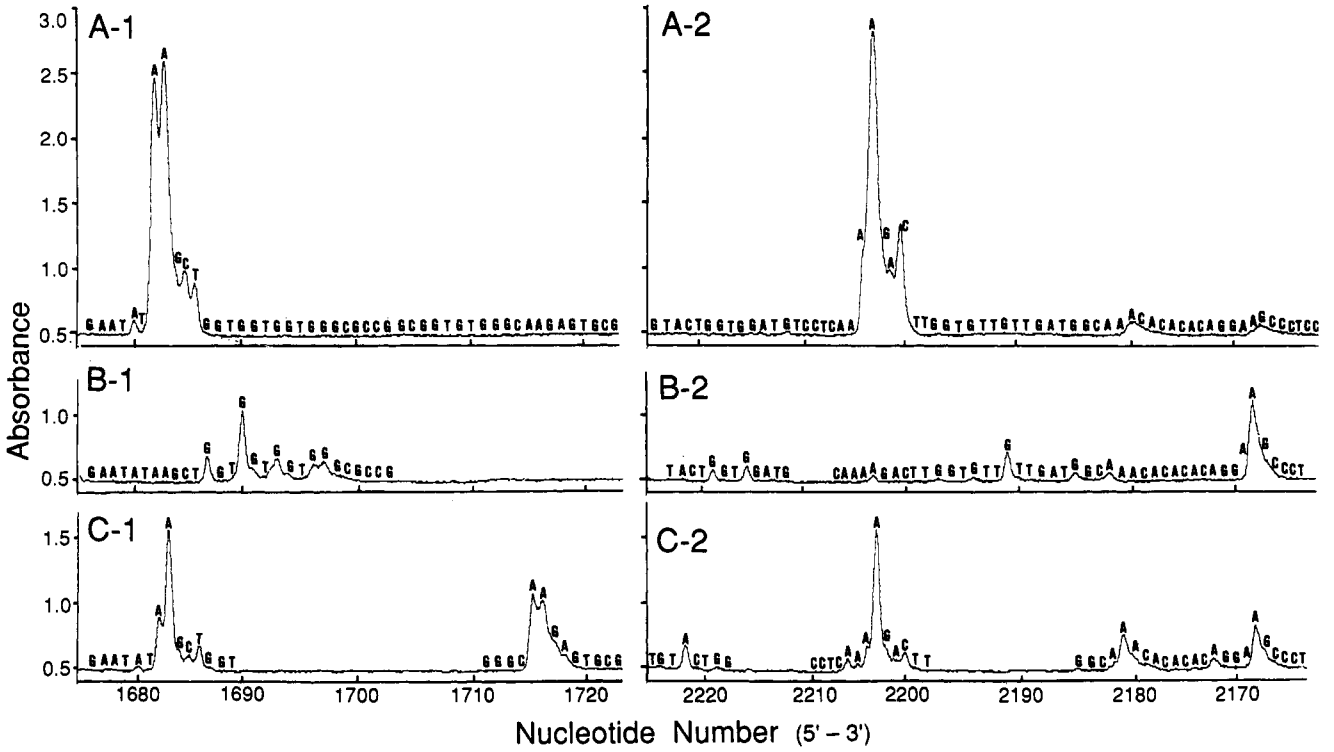

FIGURE 4: Alteration of site specificity of duocarmycin A-induced cleavage of the 261-base pair and 341-base pair DNA fragments by distamycin A or berenil. The ^{32}P 5′-end-labeled 261-base pair (1) and 341-base pair (2) DNA fragments were treated with duocarmycin A in the absence (A) or presence of DNA ligands [(B) 10 μM distamycin A; (C) 10 μM berenil] as described in Figure 1. The autoradiograms were obtained as described in Figure 2 and scanned with a laser densitometer (LKB 2222 UltroScan XL). Horizontal axis: the nucleotide number of human c-Ha-*ras*-1 protooncogene starting with the *Bam*HI site (Capon et al., 1983) (from left to right, 5′ → 3′ end).

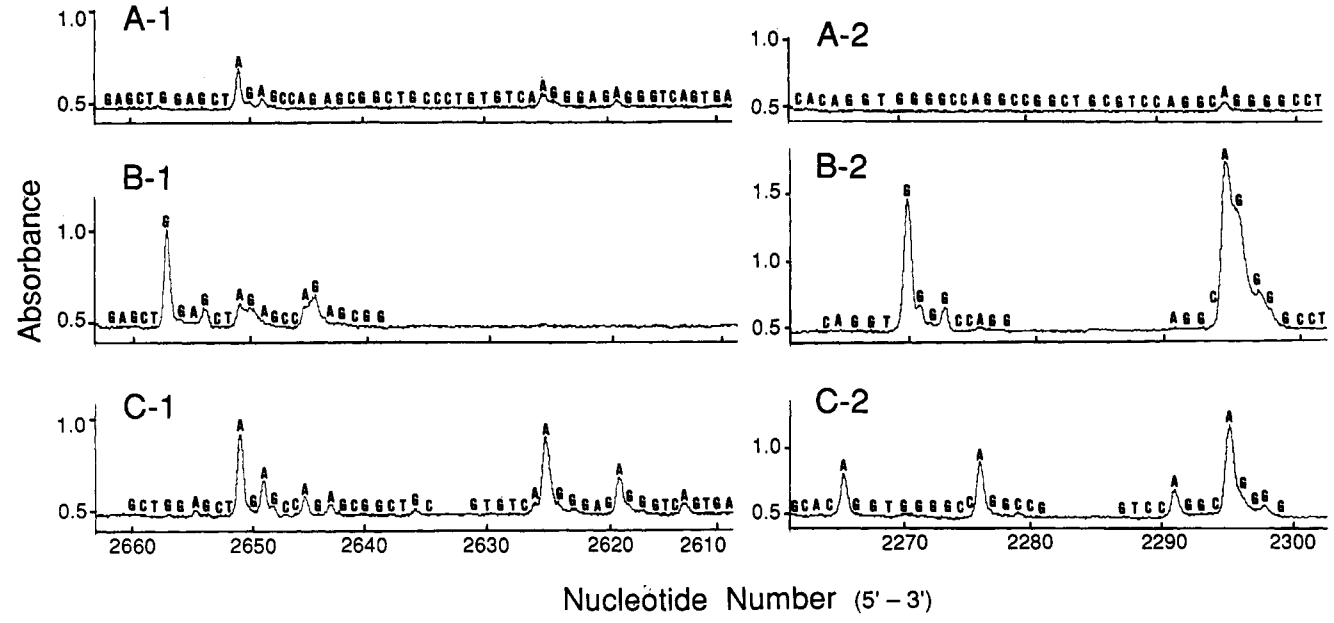

FIGURE 5: Alteration of site specificity of duocarmycin A-induced cleavage of the 337-base pair and 98-base pair DNA fragments by distamycin A or berenil. The ^{32}P 5′-end-labeled 337-base pair (1) and 98-base pair (2) DNA fragments were treated with duocarmycin A in the absence (A) or presence of DNA ligands [(B) 10 μM distamycin A; (C) 10 μM berenil] as described in Figure 2. The DNA cleavage patterns were obtained as described in Figure 4.

Guanine Alkylation of d(CCCCGGGG)$_2$ by Duocarmycin A plus Distamycin A. To confirm the novel guanine alkylation by duocarmycin A in the presence of distamycin A, HPLC product analysis was performed. The deoxyoctanucleotide, which forms a double-helical DNA octamer d(CCCCGGGG)$_2$ in neutral buffer, was incubated with duocarmycin A in the presence or absence of distamycin A and analyzed with HPLC after heat treatment. Figure 8 shows that the duocarmycin A–guanine adduct was detected when d(CCCCGGGG)$_2$ was reacted with duocarmycin A in the presence of distamycin A, whereas the adduct was not detected in the absence of distamycin A. The peak eluting at 22 min was assigned to duocarmycin A–guanine adduct on the basis of the comigration with authentic sample on HPLC under several different conditions. The authentic duocarmycin A–guanine adduct was isolated from a large-scale reaction of calf thymus DNA with duocarmycin A. The same product was also obtained by the reaction of d(GCATATGC)$_2$ or d(GCAATTGC)$_2$ with

Table I: Distamycin A- or Berenil-Dependent DNA Alkylation Induced by Duocarmycin A[a]

	Guanine Alkylation in the Presence of Distamycin A
TGGT*GGG*CGC	1696 and 1697 in 261-bp fragment; Figure 2 (lanes B1–4, bands II), Figure 4B1
TGGT*GG*TGG	1693 in 261-bp fragment; Figure 2 (lanes B1–4, band III), Figure 4B1
TGGT*GG*TGG	1690 in 261-bp fragment; Figure 2 (lanes B1–4, band IV), Figure 4B1
AGCT*GG*TGG	1687 in 261-bp fragment; Figure 2 (lanes B1–4, band V), Figure 4B1
AGCT*GG*AGC	2657 in 337-bp fragment; Figure 5B1
AGGT*GGGG*C	2270 in 98-bp fragment; Figure 3 (lanes B1–4, band IV), Figure 5B2
	Adenine Alkylation in the Presence of Distamycin A
AGGA*A*GCCC	2168 in 341-bp fragment; Figure 4B2
AGGC*A*GGGG	2295 in 98-bp fragment; Figure 3 (lanes B1–4, band I), Figure 5B2
	Guanine Alkylation in the Presence of Berenil
not observed	
	Adenine Alkylation in the Presence of Berenil
GGGC*AA*GAGT	1715 and 1716 in 261-bp fragment; Figure 2 (lanes C1–4, band I), Figure 4C1
AGCT*A*GAGC	2651 in 337-bp fragment, Figure 5C1
GTCA*A*GGGA	2625 in 337-bp fragment, Figure 5C1
GGAG*A*GGGT	2619 in 337-bp fragment, Figure 5C1
AGGC*A*GGGG	2295 in 98-bp fragment; Figure 3 (lanes C1–4, band I), Figure 5C2
GTCC*A*GGCA	2291 in 98-bp fragment; Figure 3 (lanes C1–4, band II), Figure 5C2
GGCC*A*GGCC	2276 in 98-bp fragment; Figure 3 (lanes C1–4, band III), Figure 5C2
GCAC*A*GGTG	2266 in 98-bp fragment; Figure 3 (lanes C1–4, band V), Figure 5C2

[a] The alkylated nucleotides (italics) and the adjoining four nucleotides are shown (from left to right, 5′ → 3′ end).

duocarmycin A. Characterization of duocarmycin A–guanine adduct by using dimethyl sulfate (Chan et al., 1991) revealed that duocarmycin A binds covalently through N3 of guanine. Isolation and characterization of the duocarmycin A–guanine adduct will be published elsewhere.

DISCUSSION

The present results have shown that, in the presence of distamycin A, duocarmycin A induces novel alkylation of guanine residues which are not alkylated by duocarmycin A alone. Berenil, Hoechst 33258, and DAPI altered the distamycin A-induced alkylation pattern, but alkylation of guanine was not observed. With berenil, Hoechst 33258, and DAPI, alkylation of some adenine residues was enhanced. Therefore, it is considered that berenil, Hoechst 33258, and DAPI may occupy the most preferred sites for duocarmycin A binding and consequently duocarmycin A may occupy other sites to cause DNA alkylation. Binding exclusion of duocarmycin A by these DNA ligands can explain the alteration of the alkylation pattern.

It is well accepted that distamycin A as well as berenil, Hoechst 33258, and DAPI binds in the DNA minor groove in sequences of three or more consecutive A·T base pairs. Similar to other DNA ligands, distamycin A inhibited adenine alkylation in A·T-rich sequences by "binding exclusion". On the other hand, distamycin A induced guanine alkylation. The footprinting experiment using the 98-base pair DNA fragment showed that both distamycin A and duocarmycin A bind within the sequence (5′-AGGT*GGGG*-3′). Since the sequence (5′-AGGT*GGGG*-3′) is very limited, formation of a ternary complex of the three components DNA, distamycin A, duocarmycin A) is presumed. The hypothesis of ternary complex formation was supported by HPLC product analysis and the experiment with chromomycin A_3. HPLC product analysis showed that duocarmycin A reacted with a double-helical DNA octamer $d(CCCCGGGG)_2$ in the presence of distamycin A and that an N3 guanine adduct of duocarmycin A was detected. The N3 guanine adduct formation indicates that duocarmycin A covalently binds with guanine within the minor groove of DNA. The octamer $d(CCCCGGGG)_2$, which contains neither adenine nor thymine residue, did not react with duocarmycin A in the absence of distamycin A. Relevantly, we have reported on the DNA-binding characteristics of distamycin A (Yamamoto & Kawanishi, 1992). Distamycin A enhanced bleomycin-catalyzed DNA cleavage in G·C-rich sequences. The cleavage in such a sequence in the presence of distamycin A was much greater than that in the absence of distamycin A. Neither Hoechst 33258, DAPI, nor berenil caused extensive enhancement (Yamamoto & Kawanishi, 1992). Therefore, it is considered that distamycin A induces conformational changes of DNA through interactions other than DNA minor-groove binding in A·T-rich sequences and that the altered conformation is specifically suitable for the action of drugs such as duocarmycin A and bleomycin.

It has been reported that chromomycin A_3 forms a Mg(II)-coordinated dimer [two drug molecules per Mg(II)] and concomitantly the dimer binds to G·C-rich sequences in the wider and shallower minor groove (Gao & Patel, 1990). When chromomycin A_3 plus Mg(II) was added to the reaction mixture containing the 98-base pair DNA fragment, duocarmycin A plus distamycin A-induced alkylation of 5′-AGGT*GGGG*C-3′ (2270 in 98-bp fragment) was inhibited. The result shows that the minor groove of these sequences is wide enough for binding of the chromomycin A_3 dimer and suggests that the minor groove has enough width for binding of duocarmycin A plus distamycin A. The following mechanism can be envisioned as accounting for most of the observations. Distamycin A may bind around G·C-rich sequences facilitating the binding of duocarmycin A to the relatively wide minor groove of the sequences. Ternary complex formation of DNA including the G·C-rich sequence, distamycin A, and duocarmycin A may lead to the guanine alkylation by the reactive cyclopropane ring of duocarmycin A. The cooperative drug recognition, that is, sequence-specific recognition of the DNA molecule by two different ligands in cooperation, can be designed as "concerted DNA recognition". Recent NMR studies have provided evidence for binding of distamycin A in the 2:1 mode (Fagan & Wemmer, 1992). 1-Methylimidazole-2-carboxamide netropsin, which is a crescent-shaped tripeptide like distamycin A, also binds to DNA in a similar way (Mrksich et al., 1992). In these cases, distamycin A or the netropsin derivative recognizes the minor groove of the sequences containing G·C base pairs with a

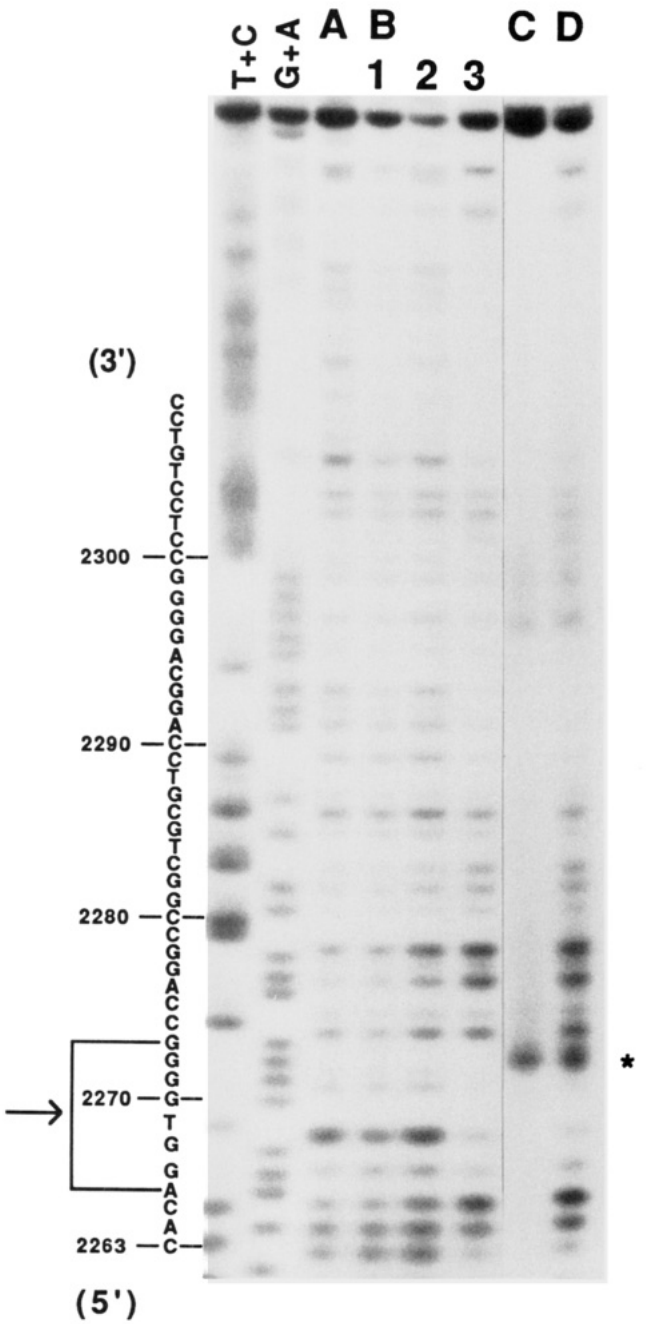

FIGURE 6: DNase I footprinting of duocarmycin A plus distamycin A bound to the ^{32}P 5′-end-labeled 98-base pair fragment. G + A and T + C lanes are for the fragment cleaved by the chemical methods of Maxam and Gilbert (1980). (A) DNase I digestion was performed in the absence of distamycin A as described in Materials and Methods. (B) DNase I digestion was performed in the presence of distamycin A at a concentration of 10 (1), 20 (2), or 50 μM (3). (C and D) The samples were pretreated with 50 μM distamycin A plus 5 μM duocarmycin A. For lane C, DNase I addition was omitted.

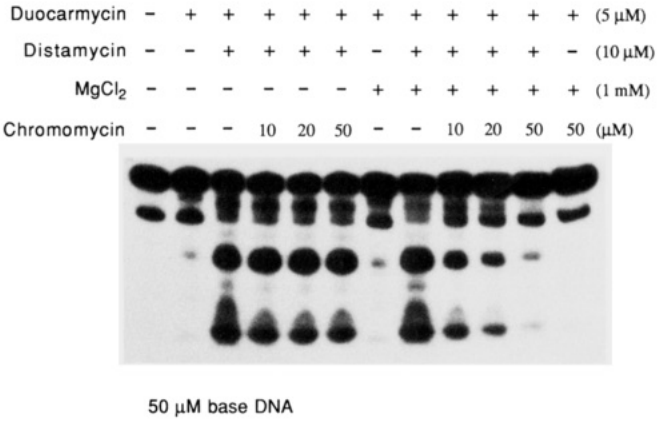

FIGURE 7: Effects of chromomycin A_3 plus $MgCl_2$ on duocarmycin A-induced DNA cleavage in the presence of distamycin A. The ^{32}P 5′-end-labeled 98-base pair fragment and sonicated calf thymus DNA (50 μM nucleotide) were treated with 5 μM duocarmycin A in the presence of 10 μM distamycin A as described in Figure 1. Where indicated, chromomycin A_3 and/or 1 mM $MgCl_2$ was added.

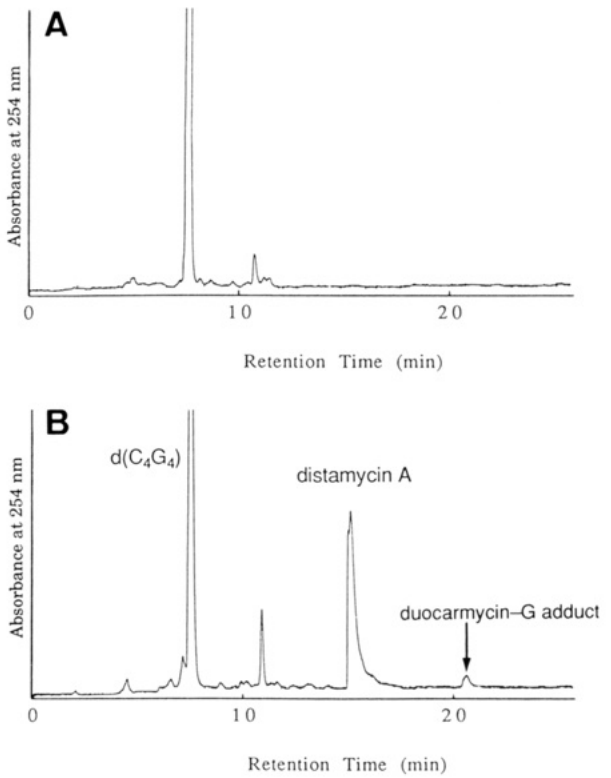

FIGURE 8: HPLC analysis of duocarmycin A-treated $d(CCCCGGGG)_2$ in the absence or presence of distamycin A. DNA octamer $d(CCCCGGGG)_2$ (1 mM nucleotide concentration) was incubated with duocarmycin A (0.1 mM) at pH 7.0 in the absence (A) or presence (B) of distamycin A (0.1 mM) at 0 °C for 1 h and analyzed after heating at 90 °C for 5 min. HPLC analysis was carried out on a Cosmosil 5C18 column as described in Materials and Methods.

side-by-side dimeric motif. The present study suggests that two different molecules (duocarmycin A and distamycin A) recognize the sequences of 5′-(A·T)G(G·C)T*G*G-3′ (italic; the novel alkylation site). Binding of a heterogeneous dimer of the two molecules to the sequences is a probable mechanism of the concerted DNA recognition.

The present results indicate that the reaction of DNA with duocarmycin A is dramatically altered with certain DNA ligands. The mechanisms of antitumor drugs acting on DNA are often studied in systems free of compounds which modulate DNA conformation. But DNA in vivo is known to interact with many biomolecules and to take various conformations. Relevantly, a CC-1065 analogue alkylates adenine of 5′-TTAGG-3′ and 5′-GTTAG-3′ sequences in vitro, while the drug was bound predominantly to nuclear DNA at sites other than the tandemly repeated sequence $(TTAGGG)_n$ in the telomeres of human chromosomes in vivo (Weiland & Dooley, 1991). The present study suggests that DNA-binding molecules causing the structural modulation of DNA are very critical for drug action and that the DNA structure in vivo can determine the mechanisms of drugs acting on DNA.

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