Conformation-Selective DNA Strand Breaks by Dynemicin: A Molecular Wedge into Flexible Regions of DNA†

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ABSTRACT: We present evidence that the enediyne antitumor antibiotic dynemicin recognizes and cleaves conformationally flexible regions of DNA. This is based on specific strand breaks at mismatches, bulges, and nicks as determined by high-resolution sequencing gels. On the basis of the weak association constant of dynemicin for DNA, it is expected that these more flexible regions would be preferred sites. In addition, the DNA unwinding behavior of dynemicin and the absorption spectrum of the dynemicin–DNA complex are strongly indicative of its intercalative binding with DNA. The results allow us to propose dynemicin as a molecular wedge that binds to the DNA by intercalating into the minor-groove side of conformationally flexible regions of DNA. Presumably, DNA local flexibility is able to create an open pocket in the minor groove, permits facile intercalation of dynemicin, and then increases the chances of its DNA damaging event. Implications for the biological action of dynemicin have also been discussed.

The enediyne families of antibiotics have become of widespread interest. Their potent antitumor activities, novel chemical structures, and fascinating modes of action elicited extensive research activities in DNA chemistry, molecular recognition, chemical synthesis, biology, and medicine (Nicolaou & Dai, 1991). Dynemicin belongs to the enediyne class of antibiotics containing a 1,5-diyn-3-ene bridging ring; however, it is distinctly unique in combining the enediyne unit with the anthraquinone chromophore of anthracycline antibiotics (Figure 1) (Konishi et al., 1990; Tokiwa et al., 1992). It is believed that dynemicin exerts its biological effect by causing DNA strand breaks. The DNA cleaving activity of the drug is significantly enhanced by reducing agents such as NADPH and thiol (Sugiura et al., 1990). The action mechanism of dynemicin involves the combined elements of those of the enediyne and anthracycline antibiotics (Figure 1) (Sugiura et al., 1990, 1991). In the first step, dynemicin A is reductively activated to the hydroquinone, which rearranges via epoxide opening to the quinonemethide followed by nucleophilic attack or protonation producing the diol or alcohol. The ring opening of the epoxide moiety shortens the distance between two acetylene bonds and then paves the way for the Bergman cyclization to generate a dehydrobenzene diradical. The reactive radical intermediate can abstract hydrogen atoms from the deoxyribose backbone of DNA (Shiraki et al., 1992), ultimately causing strand breaks and giving aromatized dynemicin H/N.

Our laboratory previously showed that dynemicin binds to the minor groove of DNA and produces sequence-preferential cleavage of DNA at the base immediately to the 3′-side of purines (Sugiura et al., 1990; Shiraki & Sugiura, 1990). However, its sequence-selectivity is relatively low when compared with other enediyne antibiotics such as esperamicin (Long et al., 1989; Sugiura et al., 1989; Uesugi & Sugiura, 1993) and calicheamicin (Zein et al., 1988, 1989). Several DNA cleavers that have low selectivity for a particular base or base-pair step exhibit specificities for certain kinds of DNA structure (Pyle et al., 1990; Burkhoff & Tullius, 1987). Indeed,

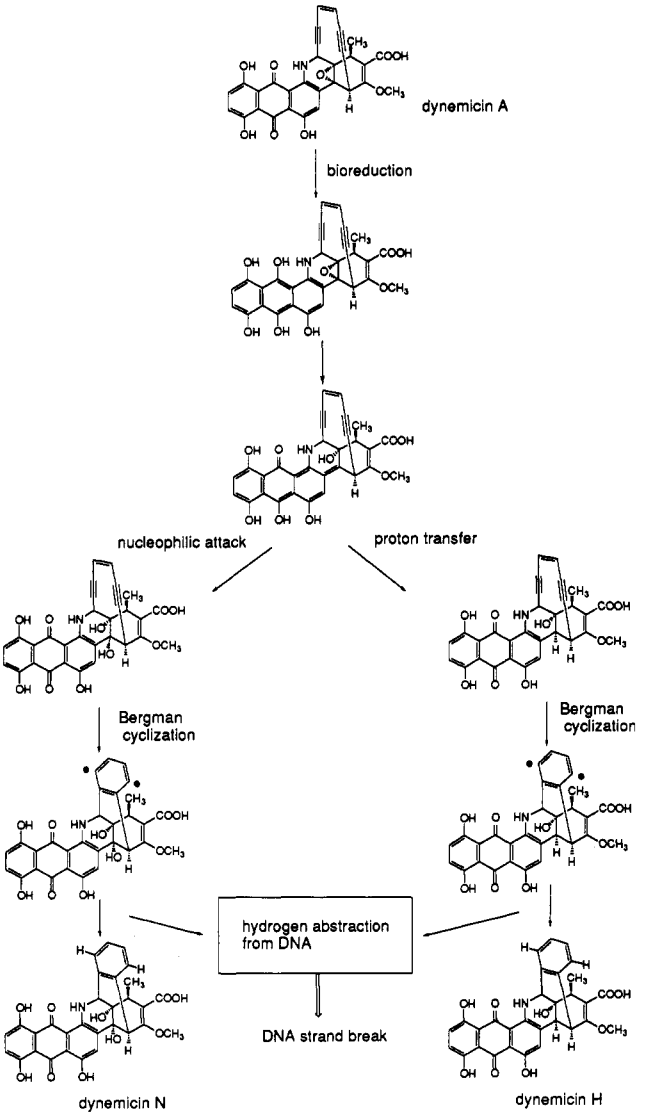

FIGURE 1: Chemical structures of dynemicins and mechanism of DNA breakage by dynemicin A.

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the DNA strand scission by dynemicin appears to be conformation-selective. The salt-induced B–Z conformational transition dramatically enhances the cleavage by dynemicin in a B–Z junction region (Ichikawa et al., 1992).

Here, we further demonstrate the conformational selectivity of dynemicin by using several oligonucleotides containing (i) a bulged base, (ii) mismatched bases, or (iii) a nicked structure. The intercalative binding of dynemicin with DNA is also clarified by monitoring its DNA unwinding behavior and by measuring absorption and fluorescence spectra of the dynemicin–DNA complex. This study reveals important characteristics of the conformation-recognition process by dynemicin. The results provide valuable information to our understanding for the biological action mode of dynemicin.

EXPERIMENTAL PROCEDURES

Drugs and Chemicals. Purified dynemicin A was kindly gifted by Bristol-Meyers Squibb (Tokyo, Japan). Dynemicin H was obtained by reaction of dynemicin A with methyl thioglycolate and checked by ^{1}H NMR features (Sugiura et al., 1991). Enzymes were purchased from Takara (Kyoto, Japan). Radiochemicals were from Amersham or DuPont. Distilled water was purified through a Sybron Nanopure II system. All other chemicals used were of commercial reagent grade.

Preparation and Labeling of Oligonucleotides. Oligonucleotides used in this study were synthesized on an Applied Biosystems 391 synthesizer. The oligonucleotides were then deprotected with ammonium hydroxide at 55 °C for 10 h and purified by HPLC on a reverse-phase column. Oligonucleotides I–III (200 pmol) were labeled at the 5′ terminal with [γ-^{32}P]ATP by using T4 polynucleotide kinase. Oligonucleotide IV (200 pmol) was labeled at the 3′ terminal with [α-^{32}P]dGTP by using the filling-in function of *Escherichia coli* DNA polymerase I large fragment. After purification on a denaturing 15% polyacrylamide gel, each oligonucleotide was heated at 90 °C for 5 min, slowly cooled to room temperature, and then allowed to reanneal at 4 °C for at least 1 h before the reaction. Under the reaction conditions of dynemicin, the expected hairpin structure of each oligonucleotide was checked by S1 nuclease digestion, 5% native gel electrophoresis, and measurements of the circular dichroism spectrum and UV melting curve. Circular dichroism spectra and UV melting curves were obtained on a JASCO J-720 spectropolarimeter and a Shimadzu UV-2200 spectrophotometer, respectively.

DNA Cleavage by Dynemicin A. A standard sample contained 50 μM dynemicin A, 400 ng of sonicated calf thymus carrier DNA, and a trace of the end-labeled oligonucleotide (<5 pmol) with 20 mM NADPH, 200 mM NaCl, 2.5 mM EDTA, and 10% (v/v) dimethylformamide in a total volume of 20 μL buffered to pH 7.5 with 25 mM Tris-HCl. The cleavage reaction was carried out at 37 °C for 3 h. To stop the reaction, ice-cold ethanol and 0.3 M sodium acetate were added, and then the DNA sample was recovered by ethanol precipitation.

Osmium Tetroxide Reaction. Each oligonucleotide was reacted with osmium tetroxide (1 mM) in a solution (50 μL) of 25 mM Tris-HCl, 2% (v/v) pyridine, 10% (v/v) dimethylformamide, 400 ng of carrier DNA, 200 mM NaCl, and 2.5 mM EDTA at 37 °C for 15 min. The modification reaction was stopped by extraction with chloroform and precipitation with ethanol. Each recovered oligomer was then treated with 1 M piperidine at 90 °C for 30 min.

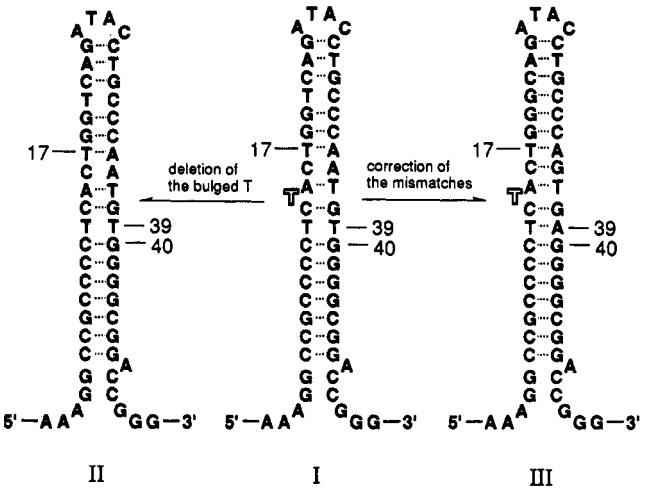

FIGURE 2: Sequences and numbering of oligonucleotides I–III used in this study. Three kinds of oligonucleotides are illustrated with the potential secondary structures. Oligonucleotide I is an imitation of the third stem–loop in the phage G4 origin of complementary DNA strand synthesis. The bulged thymidine is outlined for clarity. Oligonucleotides II and III are analogues in which the bulged thymidine is deleted and the mismatches are corrected, respectively.

DNA Sequence Analysis. Each lyophilized sample was dissolved in 3 μL of formamide containing 0.01% bromophenol blue, and loaded onto a 15% polyacrylamide/7 M urea slab gel. Electrophoresis was performed at 2000 V for 2 h. DNA sequencing was carried out by the Maxam–Gilbert method (Maxam & Gilbert, 1980). The autoradiograms were scanned with a laser densitometer (LKB Model 2222 Ultro-Scan XL).

DNA Ligation Assay. We have developed the strategy made by Montecucco et al. (1988). *Hin*dIII-linearized pBR322 DNA (0.4 μg) was equilibrated with dynemicin H at 16 °C for 30 min in the following reaction buffer (50 μL): 66 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 10 μM ATP, and 10% (v/v) dimethylformamide. Each sample was then exposed to 1.5 units of DNA ligase at 16 °C for 12 h. Before being loaded onto the gel, added drugs were removed by extraction with chloroform. Electrophoresis was performed at 4 °C and 20 V for 20 h in the presence or absence of ethidium bromide (2.3 μg/mL) by using a 1% agarose gel. After the electrophoresis, the gel was stained with 1 μg/mL ethidium bromide for 2 h and then photographed with Polaroid 665 film.

Fluorescence and Absorption Spectroscopies. Fluorescence measurements were preformed on a Hitachi F-3010 spectrofluorometer. Extinction was at 590 nm. Small volumes of concentrated calf thymus DNA were added to a solution containing 5 μM dynemicin H, 10% (v/v) dimethylformamide, and 10 mM cacodylate buffer (pH 7.2). Absorption spectra were similarly obtained with a Shimadzu UV-2200 spectrophotometer.

RESULTS

Cleavage of Oligonucleotides I–III by Dynemicin A. Oligonucleotide I mimics the phage G4 origin of complementary DNA strand synthesis, containing a stem–loop, a bulge, and mismatch structures (Figure 2) (Sugiura et al., 1990; Hiasa et al., 1989, 1990; Ueda et al., 1985). These structural features were confirmed by S1 nuclease digestion and native gel electrophoresis. The reaction with S1 nuclease was consistent with the cleavage pattern in which the loop, mismatch, and bulge domains were attacked. Native gel electrophoresis showed that the intramolecular duplex of oligonucleotide I was completely formed by annealing. Duplex formation of the oligonucleotide under the reaction conditions

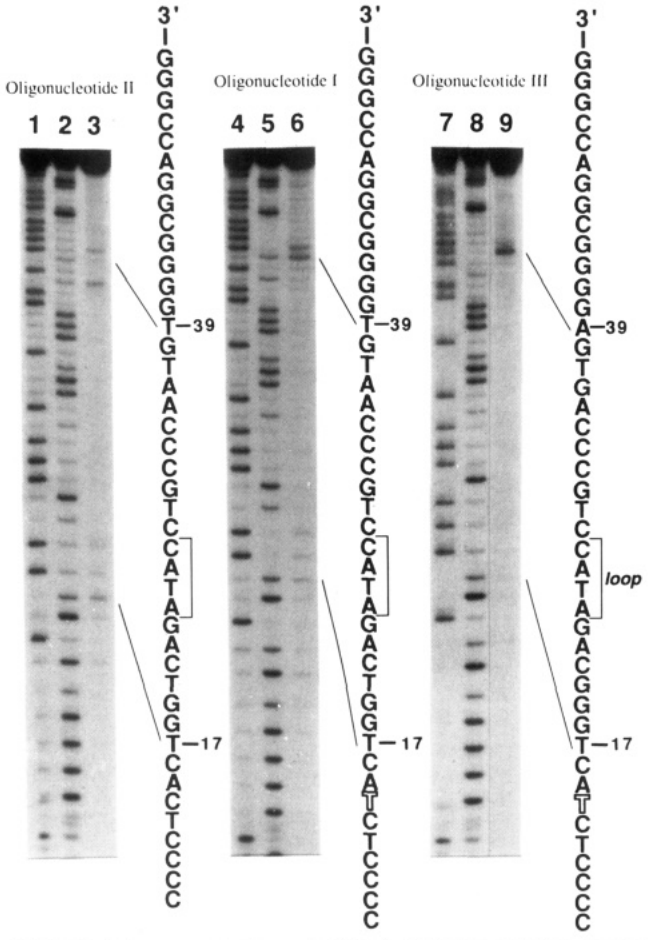

FIGURE 3: Cleavages of oligonucleotides I–III by dynemicin A. Lanes 3, 6, and 9 show the cleavage patterns of oligonucleotides II, I, and III, respectively. Lanes 1, 4, and 7 present the Maxam–Gilbert sequencing ladders for A+G, while lanes 2, 5, and 8 show the ladders for C+T. The bulged thymidine is outlined.

of dynemicin was also checked by measurements of the circular dichroism spectrum and UV melting curve.

The sites of dynemicin cleavage in oligonucleotide I were determined by using the 5′-end-labeled oligomer. As shown in lane 6 of Figure 3, dynemicin strongly cleaved oligonucleotide I at residues T-39 and G-40. Weaker cleavages were observed around T-17. In oligonucleotide II, the bulged thymidine is deleted from oligonucleotide I. The removal of the bulged thymidine significantly reduced the cleavage at T-39 and G-40, whereas the lesions around T-17 were unchanged. The marked decrease in the damage at T-39 and G-40 indicates that the introduction of the unpaired thymidine facilitates the DNA cleavage at the 3′-staggered positions on the opposite strand. In oligonucleotide III, the mismatched bases of oligonucleotide I are corrected. Despite the correction, unusually strong cleavages occurred at A-39 and G-40 in the same manner as at T-39 and G-40 in the experiment of oligonucleotide I. This result reveals that the bulge-specificity of dynemicin is independent of individual nucleotides or nucleotide mismatches in the target site. In contrast, the cleavages around T-17 disappeared, suggesting that the presence of mismatched structures is responsible for the strand breaks around T-17 in oligonucleotide I. The results in this section are summarized in Figure 4.

Reactivity of OsO_4 with Oligonucleotides I–III. OsO_4 is well-known as a specific reagent that preferentially modifies locally distorted and flexible pyrimidines in DNA (Galaska et al., 1986; Furlong et al., 1989). Figure 5 summarizes the

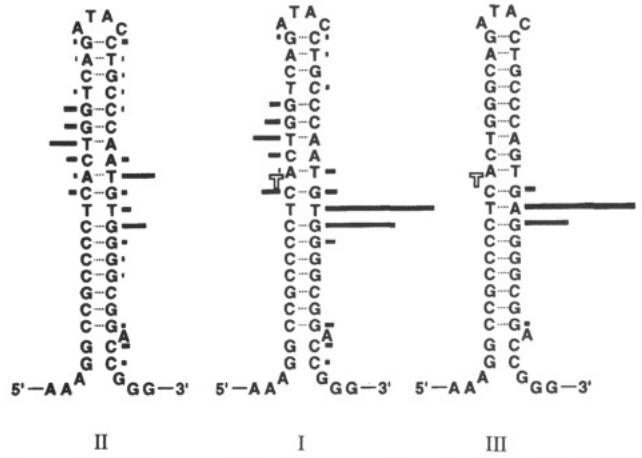

FIGURE 4: Histograms of the cleavage patterns by dynemicin A. The cleavage frequencies were obtained from densitometric scans of the gel autoradiograms shown in Figure 3. The bulged thymidine is outlined.

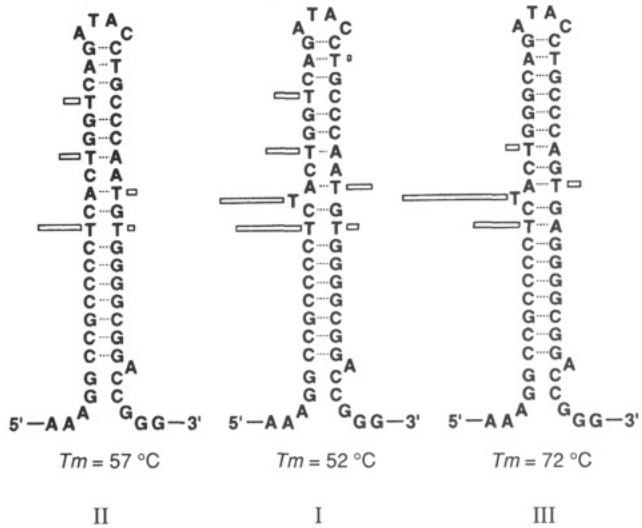

FIGURE 5: Histograms of OsO_4 modification patterns. The OsO_4 sensitive sites were visualized by treatment with 1 M piperidine. The resulting cleavage patterns were analyzed by densitometric scans. Melting temperatures (T_m) are also shown at the bottom.

relative frequencies of OsO_4 modification with oligonucleotides I–III and melting temperatures (T_m) of the oligonucleotides. In oligonucleotide I, the strong modifications were observed near the bulged thymidine and the mismatched bases. Removal of the bulge (oligonucleotide II) diminished OsO_4 reactivity in the center of the stem domain. Correction of the mismatches (oligonucleotide III) markedly reduced the modification frequencies around the mismatched sites. These observations suggest that the introductions of the bulged thymidine and the mismatched bases induces local deformation and flexibility of duplex structure near each site.

Cleavage of Oligonucleotide IV Containing a Nick Site. In order to determine the influence of DNA local flexibility on cleavage selectivity of dynemicin A, oligonucleotide IV containing a single-nick structure was tested as a substrate for dynemicin (Figure 6). Treatment of the oligonucleotide with dynemicin resulted in specific damages at residues around C-15, namely, at 3′-shifted positions from the nick on the opposite strand. Since no cleavages occurred around C-35 sites which have the same sequence without any nick, this specificity is due to local flexibility derived from the nick rather than the sequence of the target DNA. In addition, the introduction of the nick enhanced OsO_4 reactivity at residues C-14, C-15, and T-16, suggesting conformational flexibility

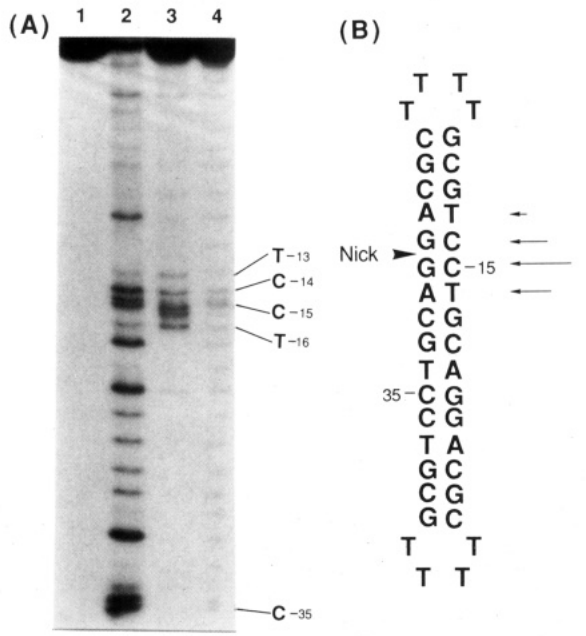

FIGURE 6: (A) Cleavage of oligonucleotide IV by dynemicin A. Lane 3 shows a cleavage pattern by dynemicin (50 μM). Lanes 1, 2, and 4 indicate intact DNA alone, the Maxam–Gilbert sequencing ladder for C+T, and the OsO_4 modification pattern, respectively. (B) Sequence of oligonucleotide IV and histogram of the cleavage pattern by dynemicin. Arrows represent the relative intensity of cleavage by dynemicin determined on the basis of densitometry.

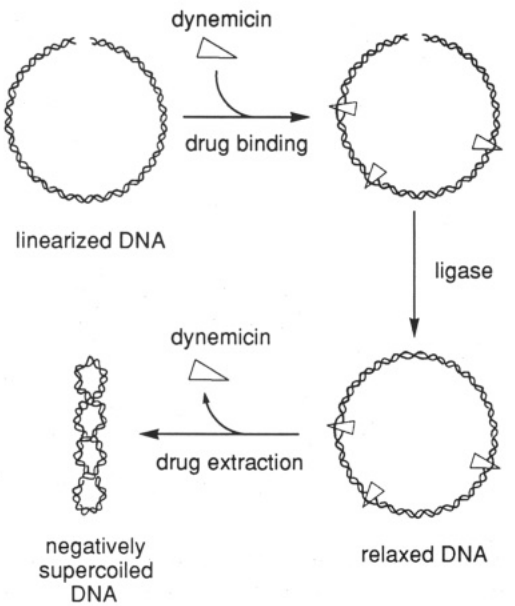

FIGURE 7: Principle of the DNA ligation assay to detect unwinding of DNA.

around the nick. These results, along with those of oligonucleotides I–III, allow us to propose that DNA cleavage by dynemicin A preferentially occurs at conformationally flexible regions in DNA.

DNA Unwinding Behavior of Dynemicin H. Figure 7 shows a principle of the DNA ligation assay to detect unwinding/winding of DNA. The substrate DNA is a linearized pBR322 DNA that lacks torsional tension even in the presence of intercalating drugs. Intercalating drugs modify the twist along the double helix (unwinding), and the consequent change in the linking number is achieved by the free rotation at the level of a linear DNA. DNA ligase converts the structure into the relaxed circular DNA. When the drug is removed, the linking number remains constant, but the twist of the DNA changes

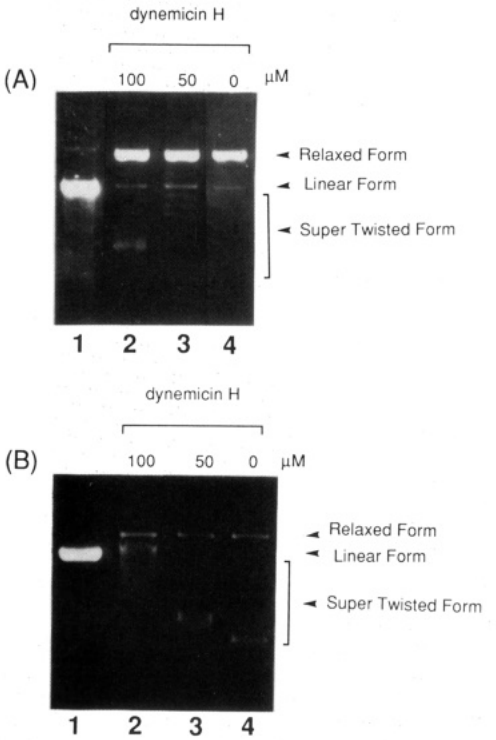

FIGURE 8: DNA ligation assay performed in the presence of dynemicin H. (A) Separation of the ligation products on an agarose gel. Linearized pBR322 molecules were ligated in the presence of dynemicin H. The drug concentration was 0 (lane 2), 50 (lane 3), and 100 μM (lane 4). Lane 1 shows unligated starting substrate. (B) Separation of the same products as in (A) on an agarose gel containing ethidium bromide (2.3 μg/mL).

to the value of the new conditions. To compensate for the resulting torsional stress, the DNA molecule negatively winds and then gives rise to negative superhelical turns.

In this assay, aromatized dynemicin H was employed, because the actual intermediate that leads to DNA strand breaks (benzene diradical form) is benzene-like rather than enediyne-like (Langley et al., 1991). Figure 8A shows the separation on an agarose gel of the products of the DNA ligation assay performed in the presence of dynemicin H. Upon ligation in increasing concentrations of dynemicin H, the resulting population of molecules became increasingly supertwisted compared with the control. In order to determine whether the DNA is unwound or tightened by dynemicin, the same samples were run on an agarose gel containing ethidium bromide (Figure 8B). Since ethidium bromide unwinds DNA, a negatively supercoiled DNA will be relaxed and shifted upward in a gel, while a relaxed DNA will be positively supercoiled and shifted downward in a gel (Snounou & Malcolm, 1983). Therefore, comparison between panels A and B of Figure 8 indicates that the ligation assay with dynemicin produces negatively supercoiled DNA, revealing the DNA unwinding behavior of dynemicin. This DNA unwinding behavior suggests that dynemicin binds to DNA by typical intercalative mode.

Changes in Fluorescence and Absorbance Spectra Induced by DNA. Addition of excess calf thymus DNA to a dynemicin H solution induced strong hypochromicity and a pronounced red shift in the absorbance spectrum (Figure 9A), consistent with intercalative binding. The discrete isosbestic point at 648 nm suggests that only one type of binding occurs under the indicated conditions. Similarly, the fluorescence of dynemicin H at 654 nm was quenched and shifted by addition of calf thymus DNA (Figure 9B). This signal provides a

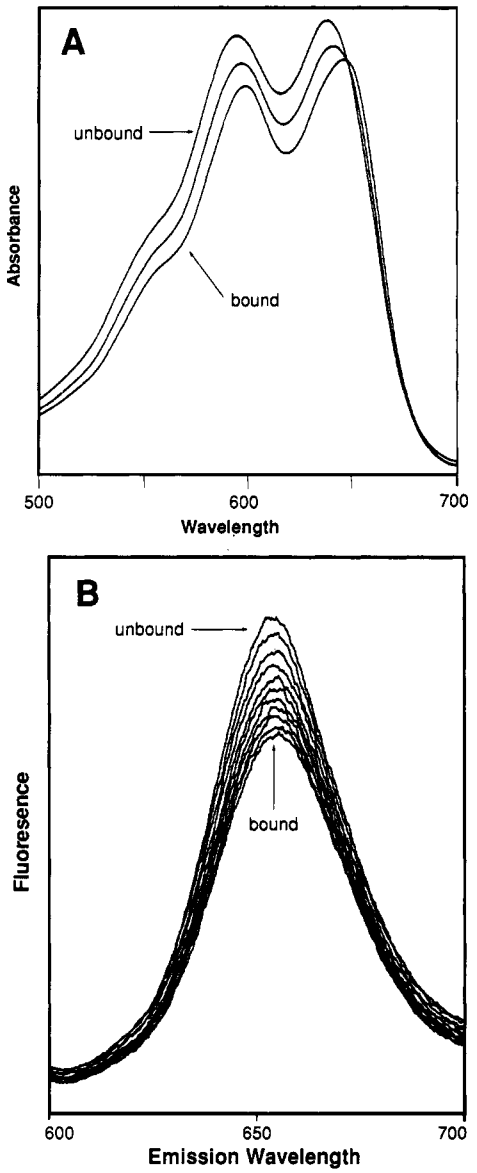

FIGURE 9: (A) Effect of DNA on the visible absorbance spectrum of dynemicin H. Calf thymus DNAs were added to a solution of 32 μM dynemicin H at pH 7.5. Final concentrations of DNA were 0, 800, and 2400 μM (bp). (B) Quenching of dynemicin fluorescence by DNA. Calf thymus DNAs were added to a solution of 5 μM dynemicin H at pH 7.5, and the measurements were made on solution ranging in concentration from 0 to 2000 μM.

window for monitoring the extent of dynemicin binding. Scatchard analysis of the fluorescence quenching data gave an apparent association constant of approximately 4.9×10^5 M^{-1} of dynemicin H for DNA.

DISCUSSION

The present experiments with the synthetic oligonucleotide substrates provide a route to examine the conformational dependence of DNA cleavage by dynemicin A. The specific strand breaks occurred at nucleotide residues near the bulge, mismatch, and nick sites. In addition, the DNA unwinding behavior of dynemicin H and spectral features of the dynemicin–DNA complex are strongly indicative of its intercalative binding with DNA.

Dynemicin Senses the Local Flexibility of the Host DNA Duplex. Insertion of the bulged thymidine into the host oligonucleotide strengthened the cleavage at the 3′-shifted residues on the opposite strand. A previous computer modeling study by Langley et al. (1991) suggested that dynemicin produces a strand break at the base to the immediate 3′-side of the intercalation site by placing the bulky benzenoid diradical in the minor groove of DNA. Therefore, the marked strand breaks at the 3′-shifted residues indicate that dynemicin is more available for minor groove intercalation at the bulge region. Similar results were observed in the studies of the phenanthroline–copper complex and neocarzinostatin, which are known to be minor groove intercalators (Williams et al., 1988; Williams & Goldberg, 1988). The location of bulge-specific scission by these compounds covers the first and second residues just on the 3′-side opposite bulged cytidine.

Previous NMR studies of d(CAAACAAAG)·d(CTTTTTTG) containing unpaired cytidine (Morden et al., 1983) and d(CGCTGAGCTCGCG)$_2$ containing unpaired thymines (Patel et al., 1987) showed that the unpaired bases bulge out from the double helix. In the case of the tridecamer d(CGCAGAATTCGCG)$_2$, two conformations are evidently possible: one with the unpaired adenosines stacked into the duplex (Patel et al., 1982) and one with the looped-out base (Joshua-Tor et al., 1988). The equilibrium between these two conformations is temperature-dependent (Kalnik et al., 1989; Pardi et al., 1982). Studies of the related 15-mer d(CGCGAAATTTACGCG)$_2$ demonstrated that the bulged bases are looped-out from the duplex in the crystal (Miller et al., 1988) but are stacked into the duplex in solution (Roy et al., 1987). These studies have clearly shown that the constraints on the bulged nucleoside are rather loose and allow considerable conformational freedom. It is quite possible that the bulge regions are relatively flexible because of the lack of hydrogen-bonding interactions. Our OsO_4 modification experiment is also indicative of local deformation and flexibility of the helix structure in the bulge region. Therefore, local flexibility of the bulge region may be able to create an open pocket in the minor groove that is accessible to dynemicin.

Mismatch and nick structures are also known to induce local flexibility in DNA which is due to lack of hydrogen-bonding interactions and fraying of the ends, respectively. Indeed, the present OsO_4 experiments show hypersenitivities of OsO_4 around each mismatch region, and the T_m value of oligonucleotide I containing mismatches (52 °C) is lower than that of oligonucleotide III (72 °C). Our results clearly demonstrate hyperreactivity of dynemicin at residues around the mismatched bases and on the 3′-side of the nick region, supporting the notion that there is a strong correlation between the local flexibility of host DNA and specific target sites of dynemicin. This proposal is consistent with our previous findings. Exonuclease digestion analysis reveals the promoted DNA binding of dynemicin under alkaline pH conditions (Arakawa et al., 1993). The salt-induced B–Z conformational transition enhances the DNA cleavage by dynemicin in a B–Z junction region (Ichikawa et al., 1992). These experimental results can be explained well in light of the conformational flexibility of the host DNA duplex. We therefore conclude that dynemicin is a molecular wedge that senses the local flexibility of DNA and causes strand breaks at flexible regions of DNA. Presumably, DNA local flexibility is able to create an open space in the minor groove, allows facile intercalation of dynemicin, and then increases the chances of its DNA damaging event.

Implications for the Biological Action of Dynemicin. Dynemicin possesses considerably potent cytotoxicity and *in vivo* antitumor activity (Konishi et al., 1989). However, the *in vitro* DNA cleaving activity of dynemicin is not so powerful as compared with those of esperamicin, calicheamicin, and

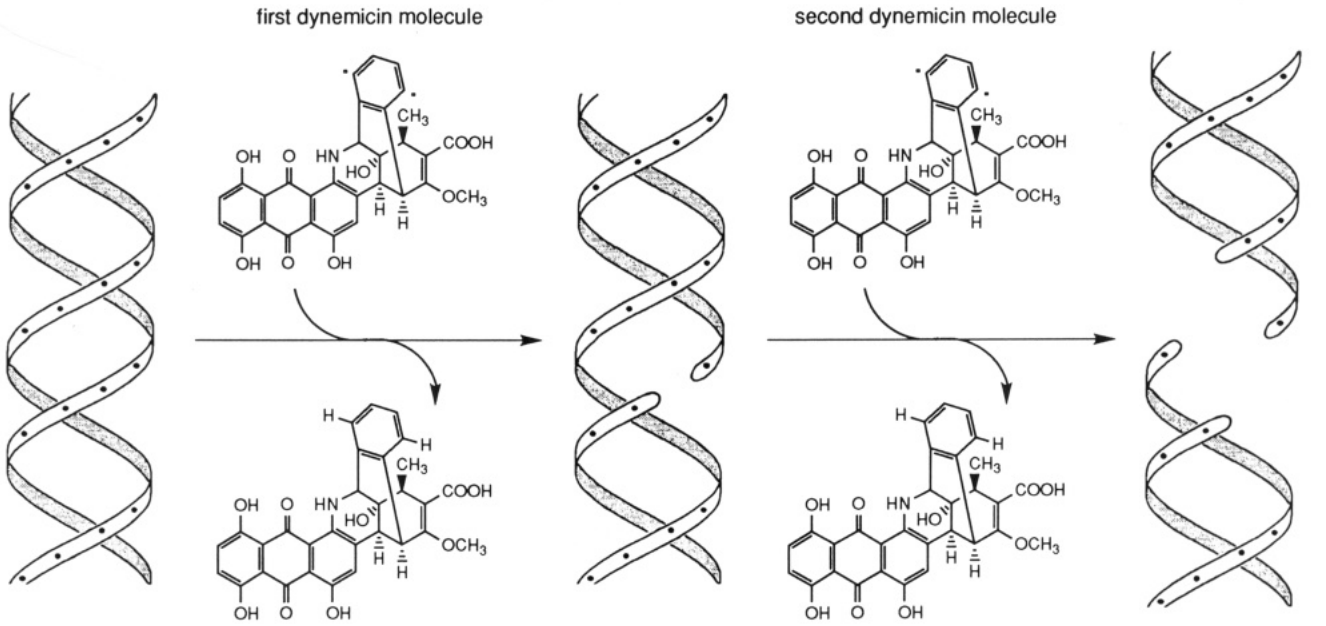

FIGURE 10: Schematic representation of the cooperative double-stranded process by dynemicin.

bleomycin (Sugiura et al., 1990). Therefore, the antibiotic may have some different factors such as damage at an essential site of DNA sequences and particular strand scission resistant to repair.

The present results evidently show the hyperreactivity of dynemicin in conformationally flexible regions in DNA. The local flexibility of the DNA conformation is believed to be an important factor in protein binding (Travers, 1989; Koudelka et al., 1988), mutagenesis, and replication of DNA (Mulder & Delius, 1972). In particular, bulge sites have been implicated as hotspots for frame-shift mutation upon replication (Streisinger et al., 1966). Therefore, the conformation-selective DNA cleavage by dynemicin demonstrated here might be related to its potent biological activity.

Furthermore, it was previously observed that dynemicin exhibits a 3′-shifted asymmetric cleavage pattern on the opposite strand, which is characteristic of double-stranded breakage in the minor groove of the DNA helix (Sugiura et al., 1990). This apparent double-stranded cleavage can result (1) from a concerted reaction involving simultaneous bi-stranded cleavage by a single drug molecule or (2) from the preferred cleavage by the second drug molecule opposite an existing single-stranded damage site. Our experiment using the nicked substrate clarified that the introduction of the nick greatly enhances selective cleavage by dynemicin at the 3′-shifted residues opposite the nick. The fact raises the latter possibility for double-stranded cleavage. Figure 10 shows a schematic representation of the cooperative double-stranded process by dynemicin. The first step is the generation of a nick site by the first dynemicin molecule. The local flexible geometry around the nick leads to a cleavage reaction of the opposite strand by the second drug molecule. As a result of the two close-spaced single-stranded breaks, an apparent double-stranded breakage ultimately occurs. Since double-stranded breaks are more difficult to repair than single-stranded breaks (Saito et al., 1973), the cooperative double-stranded cleavage by dynemicin can explain satisfactorily its potent biological activity.

REFERENCES

Arakawa, T., Kusakabe, T., Kuwahara, J., Otsuka, M., & Sugiura, Y. (1993) *Biochem. Biophys. Res. Commun. 190*, 362–370.

Burkhoff, A. M., & Tullius, T. D. (1987) *Cell 48*, 935–943.

Furlong, J. C., Sullivan, K. M., Murchie, A. I. H., Gough, G. W., & Lilley, D. M. J. (1989) *Biochemistry 28*, 2009–2017.

Galaska, G., Palecek, E., Wells, R. D., & Klysik, J. (1986) *J. Biol. Chem. 261*, 7093–7098.

Hiasa, H., Tanaka, K., Sakai, H., Yoshida, K., Honda, Y., Komano, T., & Godson, G. N. (1989) *Gene 84*, 17–22.

Hiasa, H., Sakai, H., Komano, T., & Godson, G. N. (1990) *Nucleic Acids Res. 18*, 4825–4831.

Ichikawa, A., Kuboya, T., Aoyama, T., & Sugiura, Y. (1992) *Biochemistry 31*, 6784–6787.

Joshua-Tor, L., Rabinovich, D., Hope, H., Frolow, F., Appella, E., & Sussman, J. L. (1988) *Nature 334*, 82–84.

Kalnik, M. W., Norman, D. G., Zagorski, M. G., Swann, P. F., & Patel, D. J. (1989) *Biochemistry 28*, 294–303.

Konishi, M., Ohkuma, H., Matsumoto, K., Tsuno, T., Kamei, H., Miyaki, T., Oki, T., Kawaguchi, H., VanDuyne, G. G., & Clardy, J. (1989) *J. Antibiot. 42*, 1449–1452.

Konishi, M., Ohkuma, H., Tsuno, T., & Oki, T. (1990) *J. Am. Chem. Soc. 112*, 3715–3716.

Koudelka, G. B., Harbury, P., Harrison, S. C., & Ptashine, M. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 4633–4637.

Langley, D. R., Doyle, T. W., & Beveridge, D. L. (1991) *J. Am. Chem. Soc. 113*, 4395–4403.

Long, B. H., Golik, J., Forenza, S., Ward, B., Rehfuss, R., Dabrowiak, J. C., Catino, J. J., Musial, S. T., Brookshire, K. W., & Doyle, T. W. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 2–6.

Maxam, A. M., & Gilbert, W. (1980) *Methods Enzymol. 65*, 499–560.

Miller, M., Harrison, R. W., Woldawer, A., Appella, E., & Sussman, J. L. (1988) *Nature 334*, 85–86.

Montecucco, A., Pedrali-Noy, G., Spardari, S., Zanolin, E., & Ciarrocchi, G. (1988) *Nucleic Acids Res. 16*, 3907–3918.

Morden, K. M., Chu, Y. G., Martin, F. H., & Tinoco, I., Jr. (1983) *Biochemistry 22*, 5557–5563.

Mulder, C., & Delius, H. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 3215–3219.

Nicolaou, K. C., & Dai, W.-M. (1991) *Angew. Chem., Int. Ed. Engl. 30*, 1387–1416.

Pardi, A., Morden, K. M., Patel, D. J., & Tinoco, I., Jr. (1982) *Biochemistry 21*, 6567–6574.

Patel, D. J., Shapiro, L., & Hare, D. Q. (1987) *Q. Rev. Biophys. 20*, 35–112.

Patel, D. J., Kozlowski, S. A., Marky, L. A., Rice, J. A., Broka, C., Itakura, K., & Breslauer, K. J. (1982) *Biochemistry 21*, 445–451.
Pyle, A. M., Morii, T., & Barton, J. K. (1990) *J. Am. Chem. Soc. 112*, 9432–9434.
Roy, S., Sklenar, V., Appella, E., & Cohen, J. (1987) *Biopolymers 26*, 2401–2052.
Saito, M., & Andoh, T. (1973) *Cancer Res. 33*, 1696–1701.
Shiraki, T., & Sugiura, Y. (1990) *Biochemistry 29*, 9795–9798.
Shiraki, T., Uesugi, M., & Sugiura, Y. (1992) *Biochem. Biophys. Res. Commun. 188*, 584–589.
Snounou, G., & Malcolm, A. D. B. (1983) *J. Mol. Biol. 167*, 211–216.
Streisinger, G., Okada, Y., Emrich, J., Newton, J., Tsugita, A., Terzaghi, E., & Inoue, M. (1966) *Cold Spring Harbor Symp. Quant. Biol. 31*, 77–84.
Sugiura, Y., Uesawa, Y., Takahashi, Y., Kuwahara, J., Golik, J., & Doyle, T. W. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 7672–7676.
Sugiura, Y., Shiraki, T., Konishi, M., & Oki, T. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 3831–3835.
Sugiura, Y., Arakawa, T., Uesugi, M., Shiraki, T., Ohkuma, H., & Konishi, M. (1991) *Biochemistry 30*, 2989–2992.
Tokiwa, Y., Miyoshi, S. M., Kobayashi, H., Sunaga, R., Konishi, M., Oki, T., & Iwasaki, S. (1992) *J. Am. Chem. Soc. 114*, 4107–4110.
Travers, A. A. (1989) *Annu. Rev. Bicohem. 58*, 427–452.
Ueda, K., Kobayashi, S., Sakai, H., & Komano, T. (1985) *J. Biol. Chem. 260*, 5804–5807.
Uesugi, M., & Sugiura, Y. (1993) *Biochemistry 32*, 4622–4627.
Williams, L. D., & Goldberg, I. H. (1988) *Biochemistry 27*, 3004–3011.
Williams, L. D., Thievierge, J., & Goldberg, I. H. (1988) *Nucleic Acids Res. 16*, 11607–11615.
Zein, N., Sinha, A. M., McGahren, W. J., & Ellestad, G. A. (1988) *Science 240*, 1198–1201.
Zein, N., Poncin, M., Nilakantan, R., & Ellestad, G. A. (1989) *Science 244*, 697–699.