# Some Characteristics of DNA Strand Scission by Macromolecular Antitumor Antibiotic C-1027 Containing a Novel Enediyne Chromophore†

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ABSTRACT: A new macromolecular antitumor antibiotic, C-1027, shows potent cytotoxic effects and DNA cutting activity. The DNA cleaving properties of C-1027 are compared with those of other enediyne compounds such as neocarzinostatin, esperamicin $A_1$, and calicheamicin $\gamma_1$. Even in the absence of thiols or reductants, the antibiotic C-1027 has high DNA breakage ability. Of special interest is the fact that C-1027 causes strand breaks two base pairs apart at specific sites such as 5′-TA*T*/3′-*A*TA and 5′-AG*A*/3′-*T*CT (cleavage sites in italics) in the two strands. This novel double-stranded cleavage fashion is different from that of calicheamicin $\gamma_1$, which is found to have a 3-bp separation between cleavage sites on the two strands. The asymmetric cleavage pattern to the 3′-side and a competitive experiment with distamycin A reveal minor-groove interaction of double-helical DNA with C-1027. This antibiotic appears to oxidize DNA through hydrogen abstraction predominantly at the C-4′ carbon of deoxyribose. The activation mechanism of C-1027, which contains an enediyne chromophore of the esperamicin/calicheamicin type, has been proposed.

The subject of enediyne antitumor antibiotics is currently of great interest. Antibiotics containing the unprecedented bicyclic enediyne structure are themselves converted into diradical species that attack DNA deoxyribose, and the novel mechanism of DNA damage has valuable implications for their application as potent cancer chemotherapeutic agents. Calicheamicin (Zein et al., 1989), esperamicin (Sugiura et al., 1989), and neocarzinostatin (Goldberg, 1991) are typical examples of such drugs which have high DNA cleaving ability in the minor groove. C-1027 isolated recently from a culture filtrate of *Streptomyces globisporus* C-1027 involves a labile nonprotein chromophore bound noncovalently to an apoprotein (Otani et al., 1988), and this constitution is remarkably similar to that of neocarzinostatin. However, the antibiotic C-1027 shows extremely potent antineoplastic activity against some cultured human cancer cells (Zhen et al., 1989), and indeed its cytotoxic effect is much more marked than that of neocarzinostatin. The protein moiety of C-1027 has a single polypeptide chain cross-linked by two disulfide bonds with a molecular weight of 10 500 Da, and the homology of amino acid sequences between C-1027 and neocarzinostatin is approximately 20% (Otani et al., 1991a). Although the central structure of the neocarzinostatin chromophore consists of a novel, highly strained bicyclic [7.3.0]dodecadienediyne system bearing a cyclic carbonate and an epoxide (Myers et al., 1988), the activation product of the C-1027 chromophore determined by spectroscopic methods suggests an enediyne core of the esperamicin/calicheamicin type (Minami et al., 1993). It has been demonstrated that DNA damage by neocarzinostatin results mainly in single-strand breaks, almost exclusively at thymidylic and adenylic acid residues (Takeshita et al., 1981) and that its frequency of bases attacked (T > A ≫ C > G) is clearly different from that of calicheamicin $\gamma_1$ (C ≫ T > A ≃ G) (Zein et al., 1988) or esperamicin $A_1$ (T > C > A > G) (Sugiura et al., 1989).

In the drug action of C-1027 as well as neocarzinostatin (Kappen et al., 1987), the involvement of DNA as primary target is manifested by the drug's selective inhibition of DNA synthesis in sensitive cells and by its induction of strand breaks in the DNA of mammalian cells (Sugimoto et al., 1990). This paper examines the DNA cleaving properties of a newly discovered and potent antitumor agent, C-1027. The antibiotic C-1027 shows prominent DNA cutting ability at 37 °C even in the absence of thiol compounds or reducing agents. Of interest is a novel specific double-strand cleavage pattern elicited by this molecule. This study also proposes a probable activation mechanism for the esperamicin/calicheamicin-type enediyne chromophore of C-1027. Indeed, the work characterizes the DNA cleavage of a new and very unusual natural product.

## MATERIALS AND METHODS

*Drugs and Chemicals.* Purified C-1027 was kindly supplied by Taiho Pharmaceutical Co., Ltd. (Tokushima, Japan). Plasmid pBR322 was isolated from *Escherichia coli* C600, and restriction endonucleases *Bam*HI, *Sph*I, and *Hha*I were purchased from Takara Shuzo (Kyoto, Japan). Calicheamicin $\gamma_1$, esperamicin $A_1$, and neocarzinostatin were a generous gift of G. A. Ellestad (American Cyanamid Company). T. W. Doyle (Bristol-Myers Squibb), and K. Hasuike (Yamanouchi Pharmaceutical Company), respectively. Anthramycin and distamycin A were offered by L. H. Hurley (University of Texas) and F. Arcamone (Farmitalia), respectively.

*Preparation and Labeling of DNA Restriction Fragments.* The restriction *Bam*HI–*Sph*I fragment was labeled at the *Bam*HI site. The 5′-end was labeled by using T4 polynucleotide kinase and [$\gamma$-$^{32}$P]ATP, after treatment with bacterial alkaline phosphatase (Maxam & Gilbert, 1980). The 3′-end was labeled by using [$\alpha$-$^{32}$P]dTTP and *E. coli* DNA polymerase I large fragment. After digestion with the second enzyme, *Sph*I, the singly labeled fragment was purified by polyacrylamide gel electrophoresis. The restriction *Bam*HI–*Hha*I fragment was similarly labeled at the *Bam*HI site.

*Nucleotide Sequence Analysis.* The reaction samples (total volume 20 μL) contained C-1027 (1 μM) or neocarzinostatin

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(1 μM) with the $^{32}$P-end-labeled 190-bp (*Bam*-HI–*Sph*I) pBR322 DNA fragment, sonicated calf thymus carrier DNA (5 μg/mL), and 20 mM Tris-HCl buffer (pH 7.5). The cleavage reactions were allowed to proceed with or without dithiothreitol at 37 °C for 10 min. To stop the reactions, ice-cold ethanol was added to the samples. Each lyophilized sample was dissolved in 5 μL of loading buffer containing 5 M urea, 0.1% xylene cyanol FF, and 0.05 N NaOH and then heated at 90 °C before it was loaded onto a 10% polyacrylamide gel containing 7 M urea in TBE buffer [89 mM Tris–borate and 2 mM EDTA, disodium salt (pH 8)]. DNA sequencing was carried out by the Maxam-Gilbert method (Maxam & Gilbert, 1980). The autoradiograms were then scanned with a laser densitometer (LKB Model 2222 Ultro-Scan XL).

*Identification of Double-Strand Damage Sites.* A standard sample was incubated at 0 °C for 60 min and directly taken up to electrophoresis on a native 5% polyacrylamide gel. Electrophoresis was performed at 250 V and 4 °C for 4 h. The bands were identified and cut from the gel by alignment with an autoradiogram. The DNA in a band was eluted by diffusion and resolved on a high-resolution DNA sequencing gel.

*Pretreatment of DNA with Groove Binders.* Modification of DNA with anthramycin was carried out by the method of Hurley and co-workers (Petrusek et al., 1981). In the competition experiment with distamycin A, the end-labeled DNA preparation was preincubated with distamycin A (50, 20, or 10 μM) in 20 mM Tris-HCl buffer (pH 7.5) at 37 °C for 15 min. The C-1027-induced DNA cleavage of each sample was investigated and compared with the nucleotide sequence cleavage of intact DNA with C-1027.

*Assay for Damage to Supercoiled DNA.* A standard reaction of the DNA (0.5 μg) with C-1027 (1.0 μM) was performed for 10 min in a volume of 20 μL containing 20 mM Tris-HCl buffer (pH 7.5). C-1027 was added last to start the reaction. The DNA–drug levels and the temperatures of incubation were varied as indicated in the figure captions. After ethanol precipitation, each lyophilized sample was dissolved in 20 μL of loading buffer containing 0.05% bromophenol blue and 10% glycerol. Electrophoresis was performed by using a 1% agarose gel containing ethidium bromide (0.5 μg/mL) in TBE buffer. The change of DNA forms was quantitatively estimated by the densitometer.

*Inactivation of C-1027.* In an attempt to determine the effect of DNA on inactivation of C-1027, the drug was preincubated with 2 μg of sonicated calf thymus DNA in a volume of 20 μL containing 20 mM Tris-HCl buffer (pH 7.5) for various times, and then supercoiled pBR322 DNA was added to the sample. Damage to supercoiled pBR322 DNA was examined as described above.

## RESULTS

*Effect of Temperature on DNA Cleavage by C-1027.* In contrast to neocarzinostatin, esperamicin $A_1$, calicheamicin $\gamma_1$, and dynemicin A, the antibiotic C-1027 did not require a cofactor such as thiol in the DNA strand scission (see Figure 1). Table I summarizes the temperature-dependent DNA breakage by the antitumor antibiotic. Clearly, C-1027 reveals potent DNA cutting activity even at 37 °C. Table II compares DNA cleavage ability among neocarzinostatin, esperamicin $A_1$, calicheamicin $\gamma_1$, and C-1027 at 37 °C in the absence of thiol compounds. The results indicate that (1) C-1027 has overwhelmingly high activity and (2) the DNA cutting ability decreases in the order C-1027 $\gg$ calicheamicin $\gamma_1 \simeq$ esperamicin $A_1 >$ neocarzinostatin under the present experimental conditions. In addition, C-1027 and calicheamicin

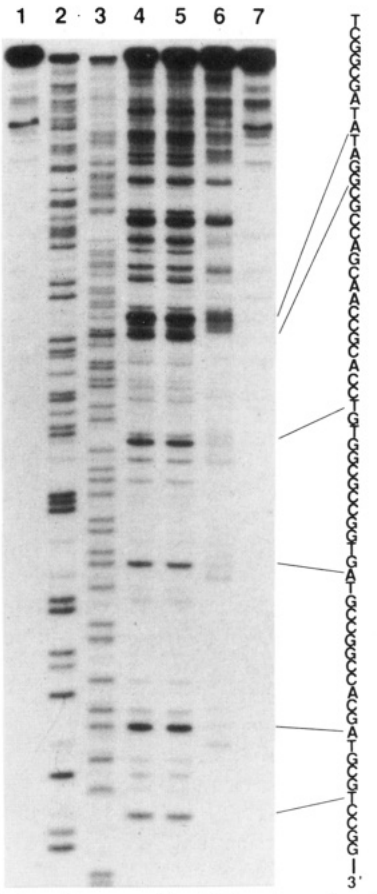


FIGURE 1: Autoradiograms of a 10% polyacrylamide/7 M urea electrophoresis slab gel for sequence analysis. 3′-End-labeled pBR322 DNA (*Bam*HI–*Sph*I fragment, 190 bp) was cleaved by C-1027 in the presence (lane 4) or absence (lane 5) of dithiothreitol. Lanes 6 and 7 compare the corresponding DNA strand scission by neocarzinostatin in the presence or absence of dithiothreitol. Lanes 1–3 show intact DNA alone, C + T, and G + A of the Maxam–Gilbert sequencing reactions respectively.

Table I: Effect of Reaction Temperature on DNA Cleavage by C-1027 (1.0 μM) at pH 7.5 for 10 Min

| | amt of DNA (%) | | |
|---|---|---|---|
| reaction temp (°C) | form I | form II | form III |
| blank (intact DNA) | 78.5 | 21.5 | 0 |
| 4 | 72.3 | 27.7 | 0 |
| 12 | 61.4 | 34.1 | 4.5 |
| 20 | 42.3 | 51.4 | 5.7 |
| 28 | 22.3 | 66.8 | 10.9 |
| 37 | 0 | 71.9 | 28.1 |
| 45 | 0 | 66.9 | 33.1 |
| 53 | 0 | 58.6 | 41.4 |

Table II: Effect of C-1027, Neocarzinostatin, Esperamicin $A_1$, and Calicheamicin $\gamma_1$ (1.0 μM) on DNA Cleavage at 37 °C and pH 7.5 for 10 Min

| | amt of DNA (%) | | |
|---|---|---|---|
| compd | form I | form II | form III |
| blank (intact DNA) | 73.1 | 26.9 | 0 |
| C-1027 | 1.8 | 70.5 | 27.7 |
| neocarzinostatin | 68.2 | 31.8 | 0 |
| esperamicin $A_1$ | 48.3 | 50.3 | 1.4 |
| calicheamicin $\gamma_1$ | 50.0 | 39.4 | 10.6 |

$\gamma_1$ produced double-strand breaks as shown in the change from form I to form III DNAs. The heat-induced (37 °C) DNA cleavage of neocarzinostatin was remarkably weak. When supercoiled DNA (0.5 μg) was incubated with C-1027

Table III: Inactivation of C-1027 in the Presence or Absence of Calf Thymus DNA[a]

| incubation time (min) | amt of DNA (%) | | | | | |
|---|---|---|---|---|---|---|
| | +DNA | | | -DNA | | |
| | form I | form II | form III | form I | form II | form III |
| 0 (intact DNA) | 72.8 | 27.2 | 0 | 72.8 | 27.2 | 0 |
| 0.5 | 16.2 | 65.5 | 18.3 | 2.9 | 69.0 | 28.1 |
| 10 | 42.1 | 49.6 | 8.3 | 4.7 | 66.2 | 29.1 |
| 30 | 64.7 | 30.4 | 4.9 | 3.2 | 68.6 | 28.2 |
| 60 | 71.8 | 26.4 | 1.8 | 2.7 | 69.3 | 28.0 |
| 90 | 73.0 | 27.0 | 0 | 3.5 | 68.1 | 28.4 |

[a] After C-1027 (1 $\mu$M) was incubated in the presence or absence of sonicated calf thymus DNA (2 $\mu$g), DNA cleavage activity was investigated at 37 °C and pH 7.5 for 10 min.

(1.0 $\mu$M) at 37 °C for 10 min in buffers of different pH, the change of DNA forms was as follows: intact DNA (form I = 79.9%, form II = 20.1%, and form III = 0%), pH 5.0 (form I = 0%, form II = 61.1%, and form III = 38.9%), pH 7.0 (form I = 0%, form II = 68.1%, and form III = 31.9%), and pH 9.0 (form I = 39.2%, form II = 46.1%, and form III = 14.7%). In the presence of thiols such as dithiothreitol and 2-mercaptoethanol, on the other hand, the DNA cleavage activities of neocarzinostatin, esperamicin $A_1$, and calicheamicin $\gamma_1$ increased significantly. By contrast, C-1027 showed similar DNA cutting ability in the presence and absence of thiols. Under the experimental conditions of 100 $\mu$M dithiothreitol at 37 °C for 10 min, the DNA cutting ability decreased in the order esperamicin $A_1 \simeq$ calicheamicin $\gamma_1 >$ C-1027 > neocarzinostatin (data not shown).

*Inactivation of C-1027 in the Presence or Absence of DNA.* The inactivation of C-1027 in the presence or absence of sonicated calf thymus DNA was investigated. In the presence of DNA, C-1027 was definitively inactivated by incubation for 30–60 min. In the absence of DNA, on the other hand, the antibiotic approximately maintained its original activity under the same conditions (Table III). The results strongly suggest that C-1027 specifically reacts with DNA and that it is moderately stable unless DNA coexists.

*Nucleotide Sequence-Specific Cleavage by C-1027.* Figure 1 presents a typical autoradiographic result with the 3′-end-labeled 190-bp pBR322 DNA fragment for DNA strand scission by C-1027 (lanes 4 and 5) and neocarzinostatin (lanes 6 and 7) in the presence or absence of dithiothreitol. A cleavage experiment for the 5′-end-labeled strand of the same DNA fragment by C-1027 was also performed, and the histograms of DNA cutting sites by the antitumor antibiotic are given in Figure 2. Dithiothreitol greatly enhanced the DNA cleavage activity of neocarzinostatin but did not elevate that of C-1027. Of special interest is the fact that C-1027 causes strand breaks two base pairs apart in the two strands. The preferred attacking sites were as follows:


Other sequences, such as 5′-CTA, 5′-TGG, 5′-AGG, and 5′-TCG and their complementary sites, were also cleaved in the same fashion. The sequence 5′-AGA as a preferential cutting site of C-1027 was also observed in the *SalI–HhaI* fragment (52 bp) of pBR322 DNA. These asymmetric cleavage patterns to the 3′ side on opposite strands indicate interaction of C-1027 with the minor groove of the DNA duplex.

Figure 3 shows an analysis of the 3′-terminal structure of the C-1027-cleaved DNA by using the 5′-end-labeled DNA fragment. The cutting bands are slightly ahead of the Maxam–Gilbert sequencing markers with 3′-phosphate termini and run identically with the DNA fragment cleaved by the iron–bleomycin system. The bleomycin–iron complex is well known to cleave DNA by C-4′ hydrogen abstraction and to form 3′-phosphoglycolate termini. The C-4′ radical of deoxyribose leads in part to the formation of 4′-hydroxylated abasic sites instead of to strand scission (Stubbe & Kozarich, 1987). As indicated in lane 3 of Figure 3, the hydrazine treatment produced several new bands migrating about one-half base slower than the Maxam–Gilbert sequencing markers. These new bands (shown by arrowheads) are presumed to be the 3′-pyridazinylmethyl terminal fragment reported in the studies on DNA cleavage by iron–bleomycin. In the 3′-end-labeled DNA fragment, on the other hand, all the C-1027-cleaved fragments possessed the same mobility as the Maxam–Gilbert

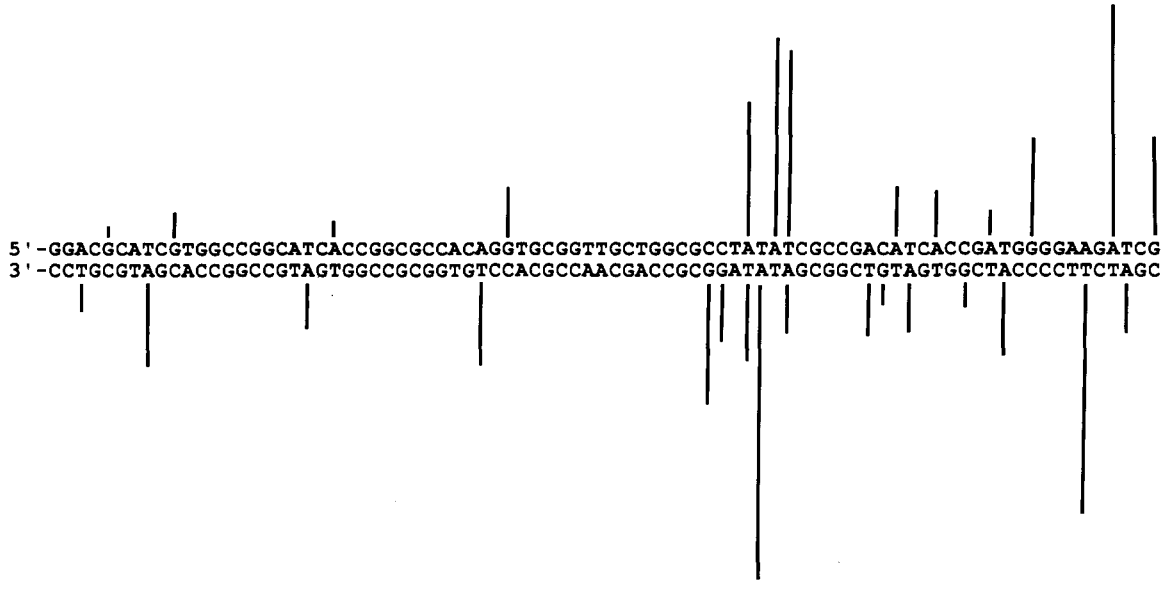


FIGURE 2: Histograms of duplex DNA cutting sites by C-1027. Relative DNA breakage frequencies were obtained from densitometric scans of the gel autoradiograms of 3′- and 5′-end-labeled DNAs. The heights of the bars represent the relative cleavage intensities at the indicated bases.

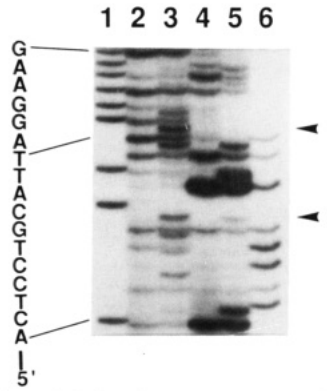


FIGURE 3: Effect of hydrazine treatment on C-1027- or iron–bleomycin-induced DNA cleavage for the 5′-end-labeled fragment. Lanes 2 and 4 present C-1027- and iron–bleomycin-induced DNA cleavages, respectively. Lanes 3 and 5 show the results of hydrazine treatment of the samples of lanes 2 and 4, respectively. Lanes 1 and 6 represent G + A and C + T of the Maxam–Gilbert sequencing reactions respectively.

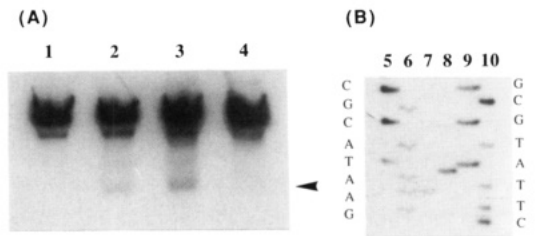


FIGURE 4: Detection of the double-stranded cleavage site. (A) The 3′- and 5′-labeled DNA fragments produced by treatment with C-1027 resolved on a native gel: lane 1, intact 3′-labeled DNA; lane 2, C-1027-treated 3′-labeled DNA; lane 3, C-1027-5′-labeled DNA; lane 4, intact 5′-labeled DNA. (B) The isolated fragment resolved on a 15% sequencing gel: lanes 7 and 8, fragments of lanes 2 and 3 indicated by the arrowhead in (A); lanes 5 and 10, C + T of the Maxam–Gilbert sequencing reactions for the 3′- and 5′-labeled DNA fragment; lanes 6 and 9, the corresponding G + A reactions.

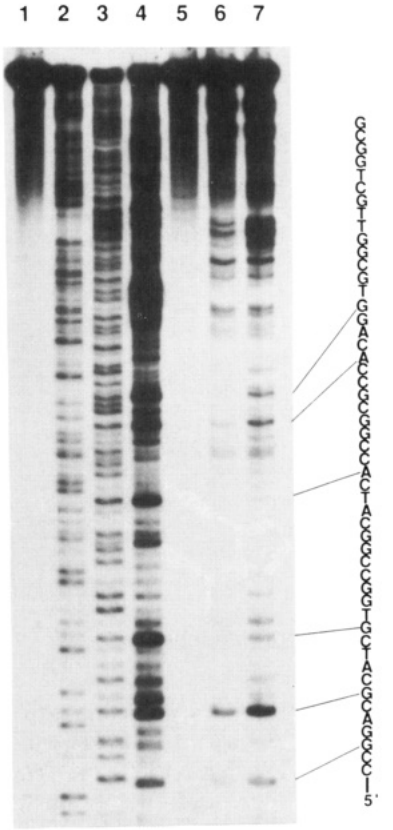


FIGURE 5: Alteration of DNA-cutting modes by C-1027 after pretreatment with distamycin A. After treatment of the 5′-end-labeled DNA fragment with 50 μM (land 5), 20 μM (lane 6), and 10 μM (lane 7) distamycin A, the DNA cleavage reactions were carried out by C-1027 (1 μM) at 37 °C for 10 min. Lane 4 shows the standard DNA strand scission by C-1027 in the absence of distamycin A. Lanes 1–3 present intact DNA alone and C + T and G + A of the Maxam–Gilbert sequencing reactions respectively.

sequencing markers, indicating that these sites have 5′-phosphate termini. The results appear to suggest that C-1027 oxidizes DNA strands through hydrogen abstraction predominantly at C-4′ carbons of the deoxyribose backbone and ultimately produces strand breakage of the DNA duplex.

*Detection of Double-Stranded Cleavage Site.* When the 3′- and 5′-end-labeled restriction fragments treated with C-1027 were electrophoresed on a native gel (Figure 4A), one double-stranded DNA fragment was detected. To determine the precise site of double-strand cutting, the double-stranded cleavage fragment (Figure 4A, arrowhead) was eluted and run on a sequencing gel (Figure 4B). As expected, the double-strand cutting site was two base pairs wide in the two strands of DNA.

*Effect of Distamycin A Pretreatment (or Anthramycin Pretreatment) on DNA Cleavage by C-1027.* Figure 5 demonstrates alteration of DNA strand scission induced by C-1027 after pretreatment of DNA with distamycin A. The C-1027-generated DNA cutting sites were clearly inhibited with increasing concentrations of distamycin A. In particular, significant protection of the C-1027 cleavage sites was detected in AT-rich regions such as 5′-CATCAC and 5′-CATCGT. At 3′-GCCGGTT and 3′-CGAGGTT sites, furthermore, modification of the guanine 2-amino group with anthramycin inhibited DNA cleavage by C-1027 (data not shown).

## DISCUSSION

*DNA Cleaving Properties and Minor Groove Interaction.* Although thiol greatly stimulates DNA damage by neocarzinostatin, esperamicin $A_1$, and calicheamicin $\gamma_1$, the antibiotic C-1027 remarkably cleaves DNA even in the absence of thiol. As clearly shown in Table I, the DNA strand scission by C-1027 is temperature dependent and even at 12 °C the DNA cutting activity is appreciably detected. Certainly, C-1027 shows potent DNA breakage at 37 °C. We previously observed that esperamicin $A_1$ effectively breaks DNA strands upon heating at 50 °C (Uesawa & Sugiura, 1991). However, the heat-induced DNA cleavage of esperamicin $A_1$ is much weaker than that of C-1027. This probably reflects the general instability of the novel enediyne chromophore of C-1027 once the protecting protein is removed.

Two striking results from this study are that (1) C-1027 induces prominent double-strand cleavage of DNA and (2) specific cleavage sites are found with 2-bp separations between the two strands. Under the present experimental conditions, C-1027 demonstrates apparent double-strand cutting. C-1027 is different from calicheamicin $\gamma_1$, which causes strand breaks 3 bp apart in the two DNA strands (Zein et al., 1988), and is similar to neocarzinostatin, which produces double-strand lesions staggered by two nucleotides. The ability of C-1027 to cause double-strand cuts at low concentration may account for its potent antitumor activity.

The preferential cutting sites of C-1027 are at adenylate and thymidylate residues, and the frequency of bases attacked ($A \geq T \gg C > G$) differs from that of other macromolecular antitumor antibiotics such as neocarzinostatin ($T > A \gg C > G$) and auromomycin ($G > T > A > C$) (Takeshita et al., 1981). This frequency is also distinct from that of other DNA-

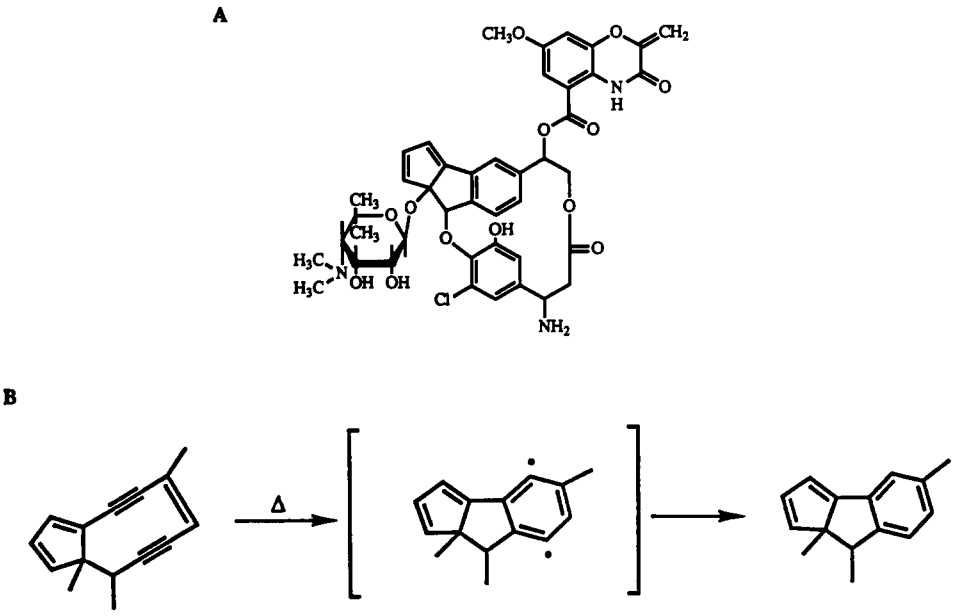


FIGURE 6: The aromatized structure of the C-1027 chromophore (A) and the probable mechanism for activation of the C-1027 chromophore (B).

cleaving antibiotics such as esperamicin $A_1$ (T > C > A > G) (Sugiura et al., 1989; Long et al., 1989), calicheamicin $\gamma_1$ (C ≫ T > A ≃ G) (Zein et al., 1988), and bleomycin (C > T > A > G) (Takeshita et al., 1981; Kuwahara & Sugiura, 1988). In addition, the major damage by C-1027 appears to be strand scission caused by hydrogen abstraction from the 4′-position of the deoxyribose of T and A residues.

Distamycin A is known to bind AT-specifically in the minor groove of DNA (Coll et al., 1987). Anthramycin reacts covalently with the 2-amino group of guanine residues and is topologically matched to the minor groove (Graves et al., 1985). Therefore, the present results strongly indicate that C-1027 interacts with the minor groove of the DNA helix. In addition, the 3′-asymmetry of the cleavage sites supports the minor groove binding of C-1027 (Sluka et al., 1987).

*Plausible Enediyne Chromophore and Activation Mechanism.* Our experiment clarified that the chromophore, isolated from C-1027 with ethyl acetate, and the parent C-1027 show identical sequence-specific cleavage patterns (data not shown). The C-1027 chromophore alone is considerably unstable (its half-life is ca. 2 h in MeOH) (Otani et al., 1991b). Although the intact C-1027 is highly reactive in the presence of DNA, C-1027 is stable in the absence of DNA. The results reveal that the protein portion of C-1027 is not contributory to the DNA cleavage but rather to protection from degradation of its chromophore. As with neocarzinostatin (Remerowski et al., 1990), the apoprotein of C-1027 presumably contains a hydrophobic cleft where the chromophore resides. It is also important to note that DNA is the specific substrate for the reaction of C-1027.

In antibiotics containing bicyclic enediyne structures, the diradical species is believed to be responsible for the DNA-damaging properties. In such rearrangements of the enediyne moiety into aromatic diradical species, it is indicated that the crucial turning point in the distance between the triple bond ends is in the range from 3.20 to 3.31 Å at room temperature (Nicolaou et al., 1988). The distance between the two triple bonds of the aglycon moiety in esperamicin $A_1$ and calicheamicin $\gamma_1$ is estimated to be 3.36 Å, suggesting that the antibiotics considerably stabilize at room temperature. Indeed, we demonstrated weak DNA strand scission of esperamicin $A_1$ at 50 °C (Uesawa & Sugiura, 1991). One degradation product derived from the C-1027 chromophore has been reported to be a 3,4-dihydro-7-methoxy-2-methylene-3-oxo-2*H*-1,4-benzoxazine-5-carboxylic acid close to the corresponding one of auromomycin (Otani et al., 1991b). The high DNA cutting ability of C-1027 at 20–37 °C appears to indicate that the enediyne chromophore of C-1027 has a shorter distance between the triple bond ends than that of neocarzinostatin, esperamicin $A_1$, or calicheamicin $\gamma_1$. Recently, a model compound has been synthesized by Doi and Takahashi that has an enediyne core of the 9-membered-ring esperamicin/calicheamicin type (distance between triple bond ends = 2.61 Å) and is easily converted into the corresponding aromatized compound at room temperature (Doi & Takahashi, 1991). On the other hand, Magnus et al. (1990) show that the release of ring strain may be more important than the distance between the reactive centers for the reactive biradical species to form. The enediyne center of C-1027 may have larger strain energy in the pseudocyclic transition state. Indeed, a recent spectroscopic study clarified that the major activation product of C-1027 chromophore is an aromatized compound, as shown in Figure 6A (Minami et al., 1993). On the basis of these results, therefore, we propose an enediyne core consisting of a 9-membered ring and a probable activation mechanism for the chromophore of C-1027 (see Figure 6B). Of special interest is the fact that the enediyne chromophore of macromolecular antitumor antibiotic C-1027 is of the esperamicin/calicheamicin type rather than the neocarzinostatin type.

In conclusion, the unusual feature of this work is that C-1027 cleaves DNA to a significant degree in the absence of reducing thiols in contrast to the other enediyne compounds. In addition, the shape and structural features joined to the apparent enediyne moiety are quite different from those of the metabolites of neocarzinostatin, esperamicin, and calicheamicin. Indeed, there are a number of unusual chemical and DNA-binding/cleavage aspects to C-1027. This work will eventually lead to new insight as to how nature uses enediynes to bind and cleave DNA.

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