DNA Sequence- and Structure-Selective Alkylation of Guanine N2 in the DNA Minor Groove by Ecteinascidin 743, a Potent Antitumor Compound from the Caribbean Tunicate *Ecteinascidia turbinata*

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ABSTRACT: Ecteinascidin 743 is one of several related marine alkaloids isolated from the Caribbean tunicate *Ecteinascidia turbinata*. It is remarkably active and potent in a variety of *in vitro* and *in vivo* systems and has been selected for development as an anticancer agent. The present study investigates the interactions of ecteinascidin 743 with DNA. Ecteinascidin 743 retarded the electrophoretic migration of both supercoiled and relaxed simian virus 40 DNA even in the presence of sodium dodecyl sulfate and after ethanol precipitation, consistent with covalent DNA modifications. Similar results were obtained in a DNA oligonucleotide derived from ribosomal DNA. However, DNA denaturation reversed the DNA modifications. The homopolymeric oligonucleotide dG/dC was modified while neither the dI/dC nor the dA/dT oligonucleotides were, consistent with covalent attachment of ecteinascidin 743 to the exocyclic amino group at position 2 of guanine. Ecteinascidin 743 was then compared to another known DNA minor groove alkylating agent, anthramycin, which has also been shown to alkylate guanine N2. Footprinting analyses with DNase I and 1,10-phenanthroline−copper and exonuclease III digestions showed that ecteinascidin 743 covers three to five bases of DNA and exhibits a different sequence specificity than anthramycin in the *Escherichia coli* tyrosine tRNA promoter (*tyrT* DNA). The binding of ecteinascidin to DNA was abolished when guanines were substituted with inosines in this promoter. A band shift assay was designed to evaluate the influence of the bases flanking a centrally located guanine in an oligonucleotide containing inosines in place of guanines elsewhere. Ecteinascidin 743 and anthramycin showed similarities as well as differences in sequence selectivity. Ecteinascidin 743−guanine adducts appeared to require at least one flanking guanine and were strongest when the flanking guanine was 3′ to the targeted guanine. These data indicate that ecteinascidin 743 is a novel DNA minor groove, guanine-specific alkylating agent.

Ecteinascidin 743 (NSC 648766) is one of several related marine alkaloids isolated from the Caribbean tunicate *Ecteinascidia turbinata* (Rinehart et al., 1990). Although extracts from this organism have been studied since the 1960's, the isolation of pure substances did not occur until 1986. Ecteinascidins are remarkably active in a variety of *in vitro* and *in vivo* systems, and ecteinascidin 743 has been selected for development as an anticancer agent.

Interactions of ecteinascidins with DNA have been proposed on the basis of X-ray crystallography and molecular modeling (Guan et al., 1993; Sakai et al., 1992). Two important observations yield clues about ecteinascidin's mechanism of action. First, the pattern of potential hydrogen bond acceptors and donors indicates that the drug is likely capable of binding to the minor groove of DNA. Secondly, ecteinascidins have a carbinolamine center at the N2 position, and elimination of the adjacent hydroxyl group results in a Schiff base vulnerable to nucleophilic attack, leading to DNA alkylation.

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The aim of the present study was to elucidate the molecular interactions between ecteinascidin 743 and DNA. Our results provide the first direct evidence that ecteinascidin 743 alkylates DNA at guanines. The ecteinascidin−DNA adducts exhibit unique properties: (1) they involve the exocyclic 2-amino group of guanine located in the DNA minor groove; (2) they require noncovalent binding of ecteinascidin 743 to duplex DNA; (3) they reverse upon DNA denaturation; and (4) their DNA sequence selectivity shows similarities and differences from the adducts produced by anthramycin.

EXPERIMENTAL PROCEDURES

Materials, Chemicals, and Enzymes. Ecteinascidin 743 (Et743) and anthramycin were provided by the National Cancer Institute Drug Synthesis and Chemistry Branch (Rockville, MD). Drug stock solutions were made in dimethyl sulfoxide (DMSO) at 10 mM, and further dilutions were made in distilled water immediately before use.

Simian virus 40 (SV40) and human c-*myc* DNA, Taq DNA polymerase, and polyacrylamide/bisacrylamide were from GIBCO-BRL (Gaithersburg, MD), the American Type Culture Collection (Rockville, MD), Perkin Elmer Cetus

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(Norwalk, CT), and New England Biolabs (Beverly, MA), respectively.

DNA Fragments and Radiolabeling. Synthetic oligonucleotides were purchased from The Midland Certified Reagent Company (Midland, TX) or synthesized using a 392 DNA/RNA synthesizer (ABI, Applied Biosystems, Foster City, CA). Phage T4 polynucleotide kinase and γ-^{32}P-ATP were purchased from Gibco BRL (Grand Island, NY) and New England Nuclear (Boston, MA), respectively. Polyacrylamide was purchased from Bio-Rad, Inc. (Richmond, CA).

The 160-base pair (bp) duplex DNA fragments containing the *Escherichia coli* tyrosine tRNA promoter (*tyrT* DNA) and guanine or inosine bases were prepared as described (Bailly & Waring, 1995).

5′-End labeling was performed by using T4 polynucleotide kinase with γ-^{32}P-ATP. Kinase reactions took place at 37 °C for 30 min in the case of the oligonucleotides or 60 min in the case of the *tyrT* DNA . They were stopped by a 10 min incubation at 70 °C. For the oligonucleotides, 5′-labeling was performed with the single-stranded oligonucleotides. Annealing was done with an excess of the complementary strand in annealing buffer (10 mM Tris·HCl, pH 7.8, 100 mM NaCl, 1 mM EDTA), heated to 95 °C for 5 min and slowly chilled to 20 °C. Excess single-stranded oligonucleotides were subsequently removed by centrifugation through a Quick Spin G-25 Sephadex column, and duplex radiolabeled oligonucleotides were stored at 4 °C (Pommier et al., 1995).

Drug Treatments and DNA Footprinting and Exonuclease Digestion. DNA fragments were reacted with drugs for 1 h at 25 °C in 40 mM NaH_2PO_4, pH 6.4, 5 mM $MgCl_2$, 0.1 mM Na_2EDTA, 15 mg of bovine serum albumin/mL, and 0.2 mM freshly added DTT. Reactions were stopped by adding 0.5% sodium dodecyl sulfate (SDS) (final concentration). For the deoxyribonuclease I (DNase I) footprinting and exonuclease III experiments, reaction mixtures were ethanol precipitated and resuspended in 10 mM Tris·HCl, pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM Na_2EDTA, 15 mg of bovine serum albumin/mL, and 0.2 mM freshly added DTT. DNase I (0.5 units in 10 μL reactions) and exonuclease III (20 units in 10 μl reactions) were then added for the indicated times. The 2:1 1,10-phenanthroline−cuprous complex with sodium ascorbate as a coreactant ($[OP]_2Cu^+$) was prepared as follows: 1,10-phenanthroline (3.6 mM) was mixed with copper sulfate (1.8 mM), and the complex was reduced to the cuprous phenanthroline form using ascorbate (9 mM) (Sigman et al., 1993). A 1 μL amount of this solution was added to 10 μl DNA reaction in 10 mM Tris·HCl, pH 7.5, and 1 mM $MgCl_2$. Reactions were stopped by adding 15 mM EDTA (final concentration) and DNA sequencing loading buffer (see below).

DNA Electrophoresis. For agarose gel analysis, 3 μL of loading buffer (1% sodium dodecyl sulfate, 0.25% bromophenol blue, 20% Ficoll, 0.1 M Na_2EDTA) was added to each sample which was then heated at 65 °C for 1−2 min before loading into an agarose gel made in TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8). Agarose gel electrophoresis was at 2 V/cm overnight.

For band shift assays using non-denaturing polyacrylamide gels, 10 μL of loading buffer was usually added to 40 μL reactions immediately before electrophoresis. Neutral loading buffer consisted of 30% glycerol, 10 mM Tris·HCl pH 7.4, 0.1% bromophenol blue, and denaturing loading buffer (DNA sequencing loading buffer) consisted of 80% formamide, 10 mM NaOH, 1 mM EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue. Non-denaturing 16% or 20% acrylamide gels were run in TBE. Electrophoresis was performed overnight at low voltage to keep gel temperature below 25 °C.

For DNA footprinting experiments, samples were precipitated with ethanol and resuspended in 2.5 μL of DNA sequencing loading buffer. Samples were heated to 90 °C and immediately loaded into DNA sequencing gels (7% polyacrylamide; 19:1 acrylamide:bis) containing 7 M urea in TBE buffer. Electrophoresis was at 2500 V (60 W) for 4 h.

Gels were dried on 3MM paper sheets, autoradiographed with Kodak XAR-2 film, and analyzed with a PhosphorImager using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

RESULTS

Binding of Ecteinascidin 743 to Duplex DNA Measured by Band Shift Assays. Incubation of SV40 DNA with increasing concentrations of ecteinascidin 743 retarded the electrophoretic migration of both supercoiled and nicked DNA. This was observed in spite of 0.5% sodium dodecyl sulfate and ethanol precipitation (Figure 1A), indicating tight binding of ecteinascidin 743 to DNA. Kinetics studies revealed that ecteinascidin 743 binding occurred progressively over a 15 min period at 37 °C (Figure 1B).

We then used a 32mer end-labeled oligonucleotide (Pommier et al., 1995) (Figure 2, bottom) to further characterize ecteinascidin 743 binding to DNA. Several conclusions can be drawn from the results shown in Figure 2. First, ecteinascidin 743 has no detectable effect on single-stranded DNA (compare lanes I−P to lanes A−H). Secondly, DNA denaturation (D in Figure 2) reverses ecteinascidin 743-induced DNA mobility effects. Additional experiments using denaturation by heat (65 °C for 20 min) or pure formamide loading buffer also showed reversal of the ecteinascidin 743-induced DNA mobility effects (data not shown). However, reversal was not as complete as with DNA sequencing loading buffer (containing formamide plus 10 mM NaOH) (see Materials and Methods). Piperidine treatment of the DNA after reversal of ecteinascidin 743 binding showed no DNA breaks, indicating no detectable depurination after ecteinascidin 743 treatment. Dissociated drug was also able to react with previously untreated DNA (data not shown). Together, these results indicate that ecteinascidin 743 interacts selectively with duplex DNA and that its binding to DNA is reversed by DNA denaturation.

The DNA sequence preference of ecteinascidin 743 was evaluated using homopolymeric duplex oligonucleotides (Figure 3A). Strong retardation was observed with the dG/dC oligonucleotide, while no detectable effect was detected either with the dA/dT or with the dI/dC oligonucleotides. The differential reactivity of ecteinascidin 743 between the dG/dC and dI/dC oligonucleotides strongly suggests that ecteinascidin 743 reacts with the amino group at position 2 of guanine from the DNA minor groove.

The selectivity for duplex DNA, reversibility upon denaturation, and evidence for involvement of position 2 of

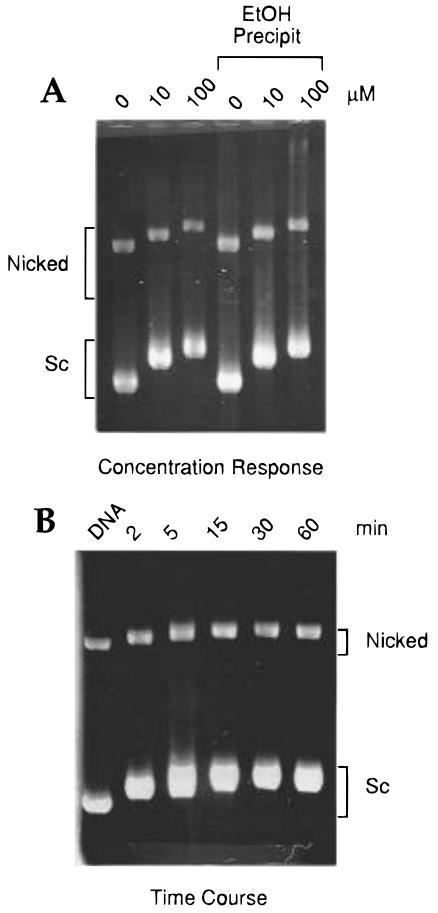

FIGURE 1: Retardation of SV40 DNA electrophoretic migration by ecteinascidin 743. (A) Concentration−response: reactions were for 30 min at 37 °C in the presence of the indicated ecteinascidin 743 concentrations. "EtOH Precipit." indicates that the corresponding samples were ethanol precipitated before electrophoresis. (B) Time-course: reactions were performed in the presence of 10 μM ecteinascidin 743 for the indicated time. In both panels, reactions were stopped by adding SDS (0.5% final concentration). "Sc" refers to the supercoiled SV40 DNA.

guanine are similar to previous findings with anthramycin (Hay et al., 1995; Kohn et al., 1974).

Comparative DNA Footprinting of Ecteinascidin 743 and Anthramycin. Since anthramycin also alkylates guanine N2 (Kohn et al., 1974; Kopka et al., 1994; Pierce et al., 1993), we compared the footprinting of ecteinascidin 743 and anthramycin using *tyrT* DNA, which is a convenient DNA fragment for drug comparison because it has been used commonly for other drug footprinting experiments and can be prepared with inosines instead of guanine (Bailly et al., 1995). Three footprinting reagents were used: deoxyribonuclease I (DNaseI) (Bailly et al., 1995; Pommier et al., 1992), the 2:1 1,10-phenanthroline−cuprous complex with hydrogen peroxide as a coreactant ($[OP]_2Cu^+$) (Sigman et al., 1993), and exonuclease III (*Exo*III) (Pierce et al., 1993). Both DNase I and $(OP)_2Cu^+$ attack the DNA from the minor groove (Sigman et al., 1993; Suck, 1994) and were expected to be sensitive to the minor groove binding of ecteinascidin 743 and anthramycin.

The DNA cleavage profiles generated with a PhosphorImager from a typical polyacrylamide gel are shown in Figure 4, along with the sequence of the *tyrT* DNA. Drug binding generally affects deoxyribonuclease I (DNase I) in two ways: protection from cleavage at the drug binding site

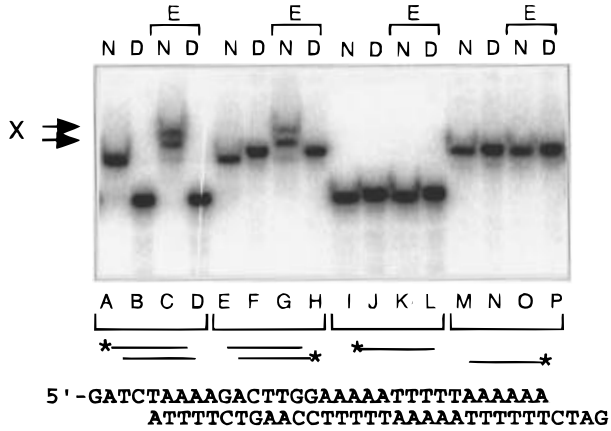

FIGURE 2: Ecteinascidin 743 adducts form only in duplex DNA and reverse upon DNA denaturation. The Topoisomerase I oligonucleotide (Fujimori et al., 1995; Tanizawa et al., 1995) was 5′-end-labeled either on the upper strand (lanes A−D and I−L) or on the lower strand (lanes E−H and M−P). Reactions were performed with either duplex DNA (lanes A−H) or single-stranded DNA (lanes I−P). Samples were either denatured (D) with DNA sequencing loading buffer or non-denatured (N) before electrophoresis in a 20% non-denaturing TBE gel. X and arrows represent ecteinascidin 743−DNA adducts.

and enhancement of cleavage in the flanks of the bound DNA (Bailly & Waring, 1995). Both ecteinascidin 743 and anthramycin protected the DNA from DNase digestion and changed the DNase digestion pattern. Each drug exhibited a different pattern (Figure 4). The drug-induced DNase I alterations were detected in the guanine-containing oligonucleotide but not in the inosine-containing oligonucleotide (data not shown), consistent with the selective alkylation of guanines by ecteinascidin 743 and anthramycin. The average distance between DNase I cleavage sites in the case of ecteinascidin 743 was three to five bases, indicating that ecteinascidin 743 protects and probably binds over approximately three to five bases in the DNA minor groove.

The 2:1 1,10-phenanthroline−cuprous complex and hydrogen peroxide as a coreactant ($[OP]_2Cu^+$) is a potent chemical reagent that also cleaves DNA from the minor groove and is sensitive to DNA conformation alterations (Sigman et al., 1993). Figure 4 (middle panels) shows the patterns of $(OP)_2Cu^+$ digestions in untreated and in ecteinascidin 743- and anthramycin-treated DNA. Both drugs changed the $(OP)_2Cu^+$ cleavage patterns. These effects were more pronounced in the case of ecteinascidin 743 than anthramycin and were only detectable in normal DNA and not in inosine-containing DNA (not shown).

Exonuclease III digests the DNA from its 3′-ends and is stopped by DNA adducts (Pierce et al., 1993). Exonuclease III is also sensitive to DNA structure, and stop points can be observed in untreated DNA (see control [C] in Figure 4). Both ecteinascidin 743 and anthramycin arrested *Exo*III. Some stop points were common to ecteinascidin 743 and anthramycin, while other were unique to each drug, indicating different effects of both drugs in *tyrT* DNA.

Together the footprinting experiments show that ecteinascidin 743 has a different base sequence preference than anthramycin and covers approximately three to five bases from the DNA minor groove.

Oligonucleotide Band Shift Assay To Determine DNA Sequence Selective Alkylation. Since ecteinascidin 743 affected DNA migration in neutral acrylamide gels (see

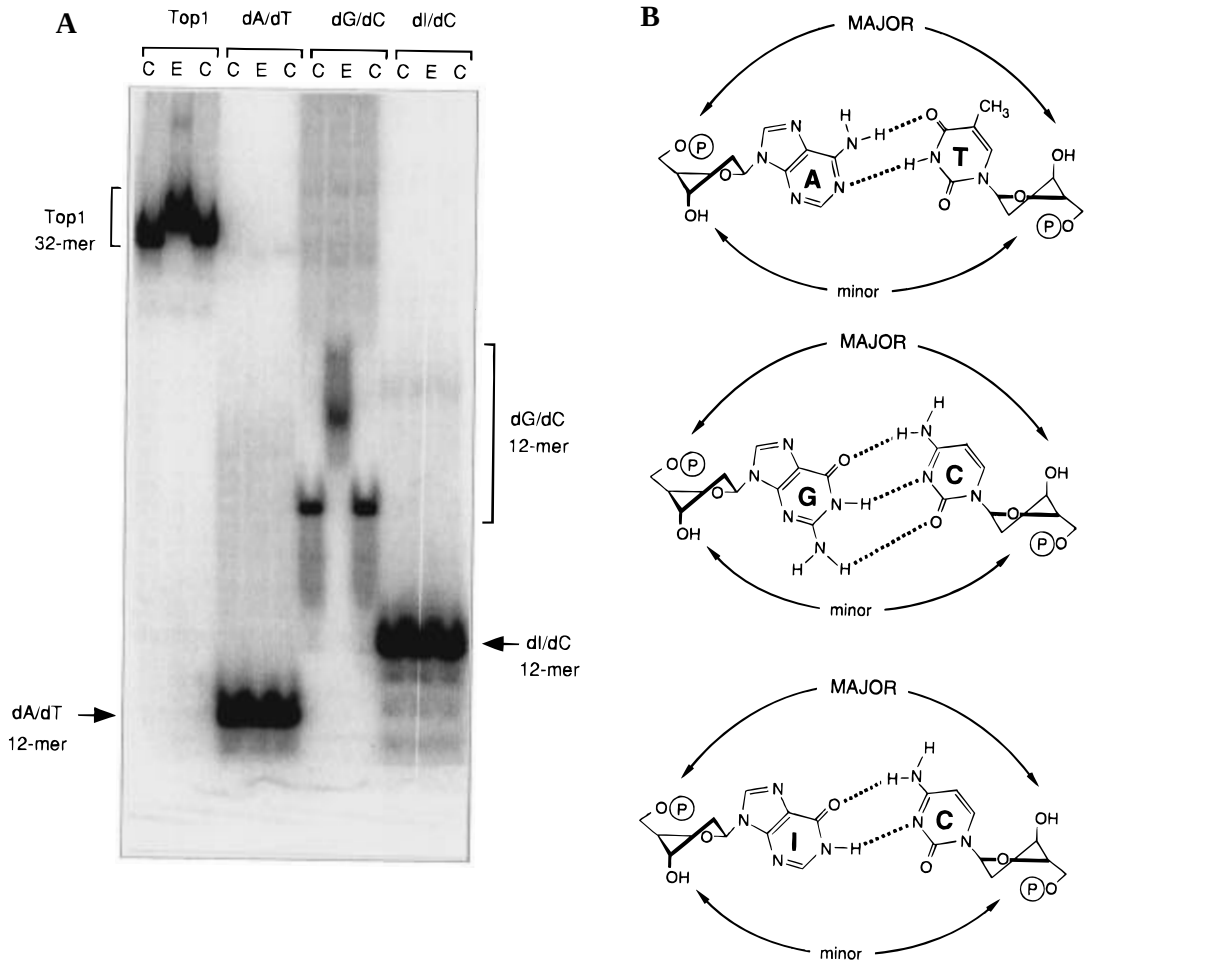

FIGURE 3: Ecteinascidin 743 forms adducts with G-containing oligonucleotides. (A) DNA electrophoresis gel picture. The oligonucleotides used are indicated at the top: Top1, 32-mer duplex oligonucleotide containing a strong top1 cleavage site (see Figure 2, bottom); dA/dT, dG/dC, and dI/dC are 10-mer homopolymer oligonucleotides. Labeling was at the 5′-DNA ends with ^{32}P. Reactions were at room temperature and were stopped after 1 h with 0.5% SDS (final concentration). Samples were loaded into a 20% non-denaturing gel. "C", control; "E", plus 10 μM ecteinascidin 743. (B) From top to bottom: structures of a dA/dT, dG/dC, and dI/dC base pairs.

Figures 2 and 3), we set up an assay using an oligonucleotide template containing a central guanine flanked by selected bases (X and Y in Figure 5, top). Inosines were used instead of guanines in the other part of the oligonucleotide to unambiguously determine the DNA sequence preference of ecteinascidin 743.

Figure 5 shows that ecteinascidin 743 retarded the DNA in a concentration-dependent manner. In the oligonucleotide shown (X = A, Y = C), ecteinascidin 743 was more potent than anthramycin and produced a more pronounced shift when compared to anthramycin. Because the shifted DNA was well-separated from the substrate DNA, it was easy to quantitate the percent of alkylated DNA by PhosphorImager analysis. Figure 6 shows some of the combinations with the three critical nucleotides to the right of each curve. Three independent experiments using GGG are represented to indicate the reproducibility of the assay. In all experiments, the GGG-containing oligonucleotide was run as a standard for calculation and the alkylated (shifted) DNA for any oligonucleotide was normalized to the GGG-containing oligonucleotide and set to 1 (Figure 7).

Ecteinascidin 743 induced most efficient alkylation in the oligonucleotides with Y being G or C, and in this set of oligonucleotides, alkylation was best in 5′-CGG and approximately half as efficient in 5′-CGC. When Y was neither G or C, then G was preferred at the X position. Presence of A or T on both sides of the central guanine resulted in almost non-detectable alkylation even at 100 μM ecteinascidin 743. Taking the 5′-XGG oligonucleotides, the efficiency of ecteinascidin 743-induced alkylation was C > T ≥ G ×c8≫ A (Figure 7). Similarly to 5′-AGG, 5′-GG(A/T) sequences were also relatively weak ecteinascidin 743 sites. 5′-CG(A/T) sequences were even less efficient with no detectable alkylation. Finally, all of the 5′-XGC sites were efficiently recognized by ecteinascidin 743, including X as A or T.

Ecteinascidin 743 and anthramycin showed similarities as well as differences in sequence selectivity (Figure 7). 5′-CGG, one of the best sequences for ecteinascidin 743, was not detectably recognized by anthramycin; and to a lesser extent, this was also true for 5′-TGG. Thus, a pyrimidine 5′ to GG appeared to enhance ecteinascidin 743 alkylation and reduce anthramycin alkylation. Conversely, 5-AGG is a poor ecteinascidin 743 site but a relatively good anthramycin site.

DISCUSSION

This paper is the first demonstration that ecteinascidin 743 targets DNA by (1) binding in the DNA minor groove in GC-rich sequences, and (2) alkylating the amino group of guanine at position 2. We also show that the ecteinascidin 743−guanine adducts can only form in duplex DNA and that they are reversible upon DNA denaturation.

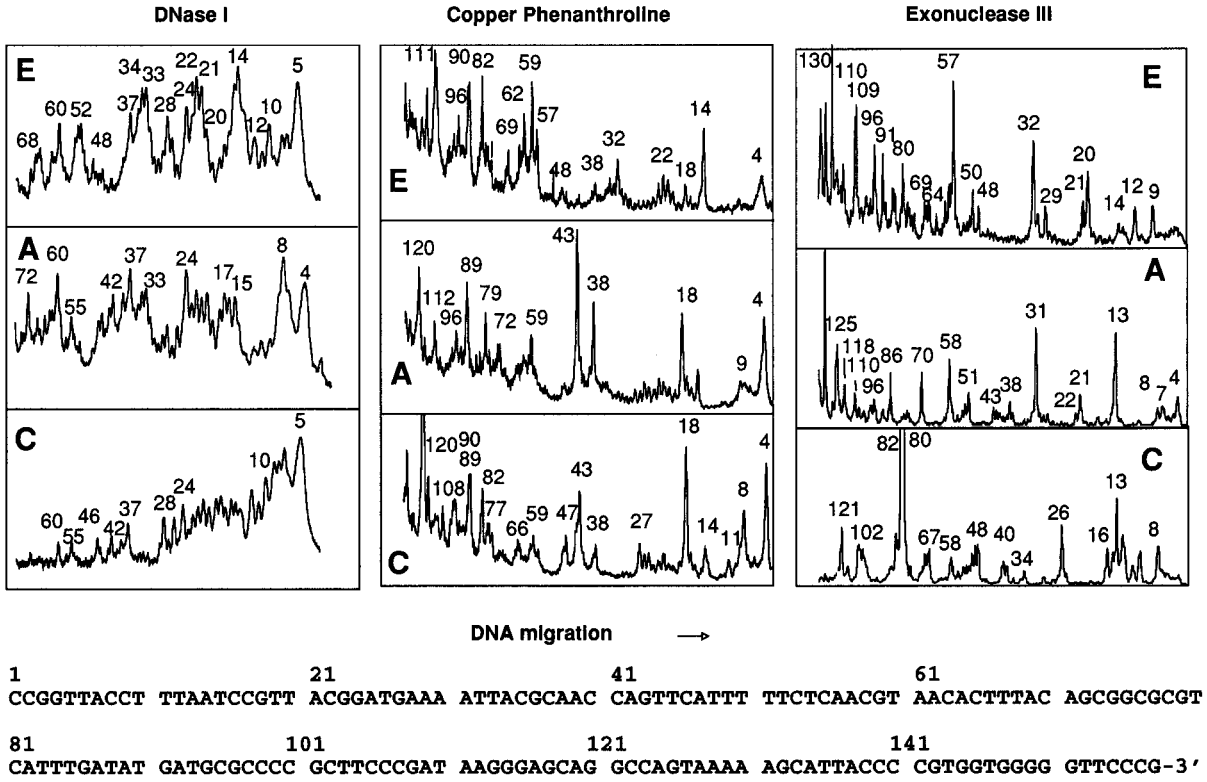

FIGURE 4: Sequence-specific alkylation of DNA by ecteinascidin 743 (E) compared with anthramycin (A) visualized as DNA cleavage intensity profiles. Lanes from a 7% polyacrylamide/urea gel were scanned using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). ^{32}P-5′-end-labeled *tyr T* DNA was reacted in the absence (control [C]) or the presence of 10 μM ecteinascidin 743 (E) or 40 μM anthramycin (A) at 25 °C for 1 h. Reactions were stopped with 0.5% SDS (final concentration) and ethanol precipitated prior to nuclease digestion. Samples were divided into 3 aliquots, each to be treated with a different footprinting reagent. Left panels: deoxyribonuclease I cleavage. Middle panels: copper−phenanthroline cleavage. Right panels: exonuclease digestion. Numbers above peaks correspond to the base positions indicated in the *tyrT* DNA sequence shown at the bottom.

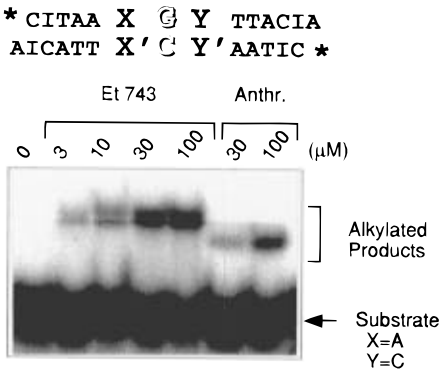

FIGURE 5: Oligonucleotide band shift assay with ecteinascidin 743 (Et743) and anthramycin (Anthr.). A series of duplex 14mer oligonucleotides (top of the figure) were ^{32}P-5′-end-labeled at both ends (asterisks). The bases flanking the central G (X and Y) were permuted. Reactions were at room temperatures and were stopped after 1 h with 0.5% SDS (final concentration).

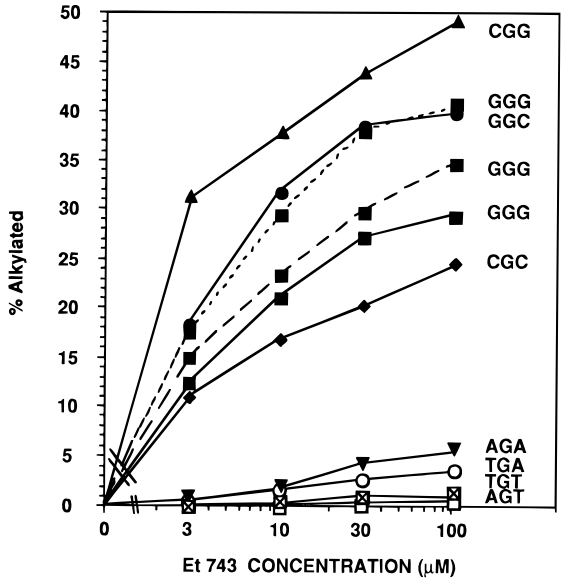

FIGURE 6: Quantitation of oligonucleotide band shift assays to measure DNA sequence selective guanine alkylation by ecteinascidin 743. The assays were performed with the oligonucleotide containing the central three bases indicated to the right of each curve (see oligonucleotide and assay in Figure 5). Examples of three independent experiments using the GGG oligonucleotide are shown (black squares with solid, dashed, and dotted lines).

At the present time, the most widely used DNA alkylating agents in cancer chemotherapy are cisplatin, nitrogen mustards, and halogenated nitrosourea, which form N7 or O6 guanine adducts from the DNA minor groove with relatively limited DNA sequence selectivity (Kohn et al., 1988). Several classes of minor groove binders have been extensively characterized. Distamycin, netropsin, and Hoechst derivatives are reversible minor groove binders selective for stretches of four to six consecutive A/T bases. Lexitropsins have also been developed to obtain more GC-selective minor groove binders (Lown, 1994). AT specific minor groove alkylating agents include CC1065 and duocarmycins (Boger & Johnson, 1995). They differ from ecteinascidin 743 for they alkylate adenine at N3 and bind in the minor groove in the 5′ → 3′ direction from the alkylation site covering 4−5 bp across an AT-rich region (Boger & Johnson, 1995). More closely related to ecteinascidin 743 are the other guanine

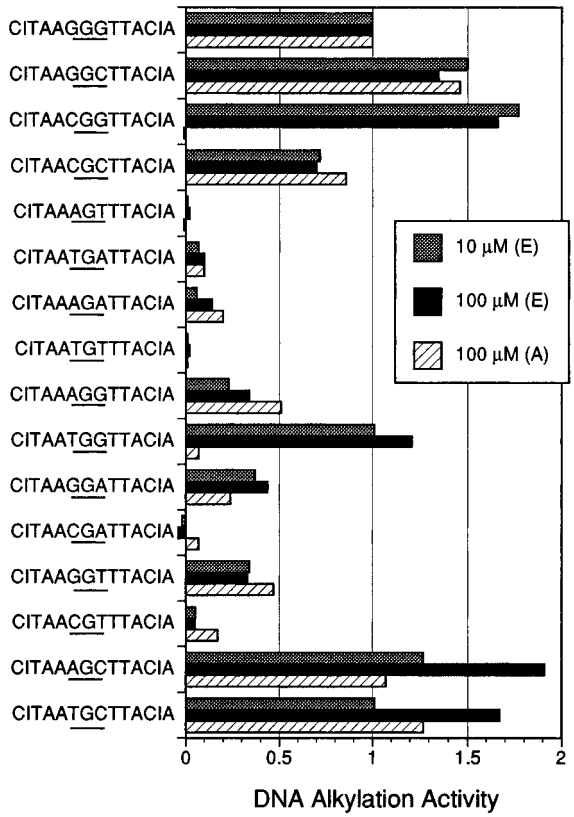

FIGURE 7: DNA sequence selective guanine alkylation by ecteinascidin (E) compared to anthramycin (A). The upper strand of the oligonucleotides used (Figure 5) are indicated to the left with the central variable triplet underlined. DNA alkylation activity was measured by the oligonucleotide band shift assay (see Figures 5 and 6) normalized to the alkylation produced in the GGG-containing oligonucleotide (top) which was run as a standard in each experiment.

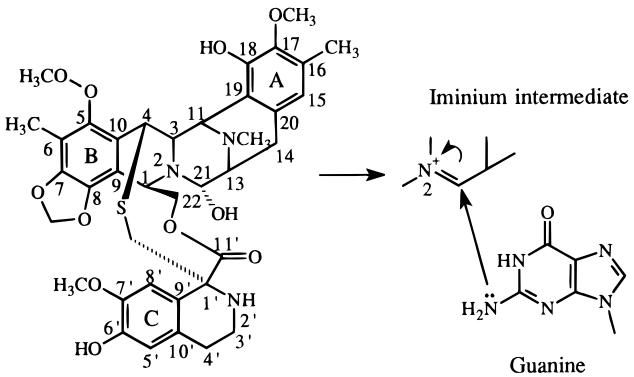

FIGURE 8: Chemical structure of ecteinascidin 743 and proposed reaction of its reactive iminium intermediate with the guanine 2-amino group.

N2 alkylating agents, mitomycin C and benzodiazepines from various strains of *Streptomyces*: anthramycin, sibiromycin (from Siberia), tomaymycin (from the Tomei region in Japan), neothramycins A and B, and saframycin (Kopka et al., 1994; Pierce et al., 1993). The most important functional group for the antitumor activity of these molecules is a carbinolamine, which is also found in ecteinascidin 743 (N2−C21−OH [Figure 8]) and several other antibiotics such as saframycins, safracins and naphthyridinomycins (Guan et al., 1993). All of these antibiotics probably share the similar mode of DNA alkylation: formation of covalent adducts between the drug and the DNA, with the target site being the exocyclic 2-amino group of guanine located in the DNA minor groove.

In contrast to other groove-binding drugs that interact with DNA within seconds, the reaction of ecteinascidin 743 with DNA took minutes to complete (Figure 1), which is also the case of anthramycin (Hay et al., 1995; Kopka et al., 1994). This may be interpreted as the slow formation of a reactive iminium ion intermediate (Figure 8), similar to the formation of a reactive imine proposed in the case of anthramycin (Kohn et al., 1974; Lown & Joshua, 1979). We found the ecteinascidin 743 adducts to be reversible upon DNA strand separation. A similar reversibility was previously noted for anthramycin, and both the formation and reversal rates were found to be acid catalyzed (Hay et al., 1995; Kohn et al., 1974). These results are consistent with the docking of ecteinascidin 743 in the DNA minor groove and positioning of the C21 atom of ecteinascidin 743 for efficient nucleophilic attack from guanine N2. Hence, the DNA contributes to both the physical (binding) and chemical (covalent bond formation) steps in the formation of ecteinascidin 743 C21−guanine N2 adducts. The structure of ecteinascidin 743 (Figure 8) has been resolved by Wang and co-workers after crystallization (Guan et al., 1993; Sakai et al., 1992). These authors have also modeled the drug−DNA interactions and predicted the guanine N2 alkylation observed in the present study, as well as a 3−4-base interaction, which is what we are observing in our DNase I footprinting experiments. Our finding that the bases immediately flanking the alkylation site are selective for ecteinascidin 743 and differ from the preferred bases flanking anthramycin alkylation sites is also consistent with this model. Our data using the oligonucleotide band shift assay showing maximum alkylation at GC-rich triplets sets ecteinascidin 743 apart from anthramycin (Guan et al., 1993) and even further apart from other minor groove alkylators which selectively react with AT-rich sequences, such as CC1065 and duocarmycins (Boger & Johnson, 1995). The crystal data of Wang and co-workers (Guan et al., 1993) show that ecteinascidin 743 can be subdivided into three components: rings B and C (see Figure 8) which each interact with one side of the minor groove and ring A which is almost perpendicular to these two rings across the minor groove. A set of five H-bonds stabilizes the ecteinascidin 743 interaction in the minor groove with the three to four preferred bases. The differences between ecteinascidin 743 and anthramycin reside in the set of noncovalent interactions which are translated into different base preferences around the alkylated guanine. These differences were also obvious from the exonuclease III, copper−phenanthroline, and deoxyribonuclease assays.

Ecteinascidin 743 is being evaluated for development as an anticancer agent because of its potency and activity in several animal models (Guan et al., 1993). Our study demonstrates its unique mechanism of action: guanine N2 alkylation from the DNA minor groove at GC-rich sequences. Data from the National Cancer Institute Drug Discovery Screen using the tumor cell line panel indicate the high potency of the compound and a unique profile of activity when compared to standard agents presently used in cancer chemotherapy. Hence, it is plausible to expect that this novel mechanism of action will be translated into a novel spectrum of anticancer activity. Ecteinascidin 743 is also an interesting

reagent. By alkylating the DNA in the minor groove, it can be used to probe DNA ligands (drugs or proteins) in GC-rich sequences. The main advantage of ecteinascidin as a probe is the reversibility of the adducts upon DNA denaturation. Hence, alkylation and footprinting analysis can be carried out under physiological conditions and the reaction products can be directly analyzed after standard denaturing gel electrophoresis.

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