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A Noncovalent Binding-Translocation Mechanism for Site-Specific CC-1065-DNA Recognition

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ABSTRACT. The molecular strategy by which small organic compounds recognise specific DNA sequences is of primary importance for rational drug design. CC-1065 is a potent alkylating agent that binds covalently to N3 of adenine and lies in the minor groove of double-stranded DNA. Its reaction with DNA occurs in a site-specific manner, with a preference for A · T-rich nucleotide sequences. In the present study, we developed a drug translocation assay to investigate the mechanism underlying this sequence selectivity. After exposure of plasmid DNA to saturating amounts of CC-1065, we observed that nearly 70% of plasmid-bound CC-1065 molecules formed stable, but noncovalent, complexes with DNA. These noncovalently bound drug molecules resisted purification by ethanol precipitation, dialysis, and sucrose gradient centrifugation, but retained the ability to translocate to DNA fragments containing a single high-affinity site for alkylation. This combination of noncovalent binding interactions and drug translocation provides a mechanism by which CC-1065 may locate specific alkylation sites in DNA. BIOCHEM PHARMACOL 52;3:447–453, 1996.

KEY WORDS. minor groove; DNA alkylation; sequence specificity; noncovalent binding; translocation; anticancer therapy

CC-1065 is a natural antibiotic and genotoxic agent produced by *Streptomyces zelensis*. It consists of 3 repeated pyrroloindole subunits (A, B, and C in Fig. 1), of which subunit A contains a DNA-reactive cyclopropane function [1, 2]. CC-1065 is the lead compound for a series of sequence-selective drugs that have entered clinical trials for use in anticancer therapy [3, 4]. Under experimental conditions, CC-1065 displays antineoplastic activity against a broad spectrum of tumours, but the clinical development of this agent was precluded by its high toxicity in mammalian systems [2]. In subsequent studies, various synthetic derivatives of CC-1065 have been developed and shown to maintain antitumour activity with significantly reduced toxicity [5, 6].

CC-1065 exterts its biological effects by reacting covalently with double-stranded DNA, thereby interfering with essential pathways of DNA metabolism [7]. Previous studies established that CC-1065 binds to N3 of adenine and lies within the minor groove of the double helix [8]. The preferred drug binding site is an A · T-rich sequence extending over 5 base pairs, with an absolute requirement for adenine at the 3′ end of this sequence. The covalent CC-1065-DNA adduct is stabilised by noncovalent interactions derived from van der Waals and hydrophobic forces, overlapping 4 base pairs to the 5′-side of the covalently modified adenine [8].

DNA alkylation by CC-1065 is a multistep process, whereby noncovalent interactions with the minor groove precede covalent bond formation [1, 2, 9]. Several lines of investigation have used synthetic derivatives of CC-1065 to elucidate the mechanistic details of its sequence-selective reaction with DNA, but these studies yielded contrasting results. In particular, the possible contribution of noncovalent drug-DNA binding forces in mediating the DNA sequence selectivity of CC-1065 is disputed. One line of experimentation indicated that site selectivity is primarily controlled by the alkylation function of subunit A, and noncovalent interactions between DNA and subunits B and C only modulate this specificity by increasing the chemical reaction kinetics [9]. This model was challenged by comparing the site selectivity of natural and unnatural enantiomers of CC-1065 and related compounds [10, 11]. These latter experiments led to the opposite conclusion, that site specificity is primarily controlled by preferential noncovalent binding into the minor groove and not by the subsequent alkylation reaction [1, 10, 11]. In the present study, we have developed a drug translocation assay to analyse the possible role of noncovalent CC-1065-DNA interactions. Our results indicate that these noncovalent binding forces may facilitate site-specific DNA alkylation by a molecular "hopping" mechanism.

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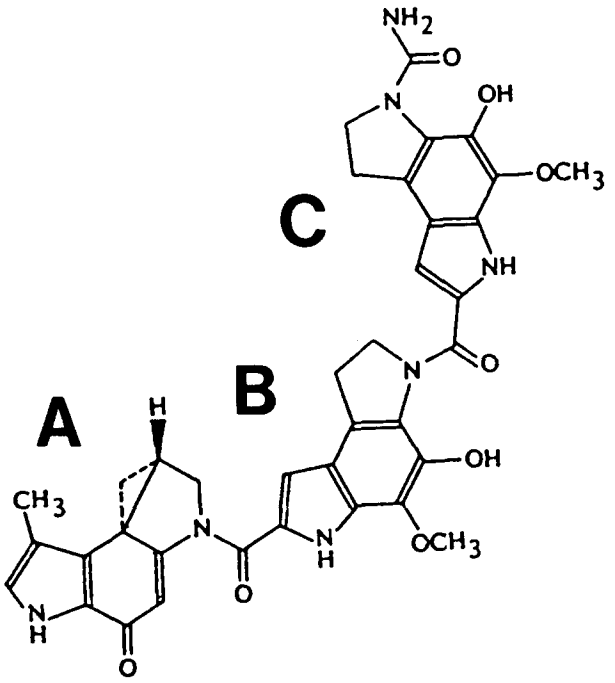

FIG. 1. Structure of CC-1065. This antibiotic molecule consists of 3 pyrroloindole subunits, of which subunit A contains a DNA-reactive cycloproyl centre.

MATERIALS AND METHODS

Materials

Compound CC-1065 was generously provided by Dr. J. P. McGovren (The Upjohn Company, Kalamazoo, MI, U.S.A.). Stock solutions of this chemical were prepared in DMSO and stored at −80°C. Plasmids pUC19 and polynucleotide kinase were purchased from Gibco BRL. Synthetic oligonucleotides were from MWG-Biotech and purified by polyacrylamide gel electrophoresis [12]. [γ-^{32}P]ATP (3000 Ci/mmol) was from Du Pont-New England Nuclear. All other chemicals used were of the highest purity commercially available.

Drug-DNA Complexes

Plasmid pUC19 was prepared by alkaline lysis from *Escherichia coli* strain DH5α, grown without chloramphenicol amplification, and purified by CsCl centrifugation [12]. Plasmids containing an average of 103 CC-1065/molecule were prepared by incubating pUC19 (46 μg/mL) with 8.6 μM CC-1065 (molar nucleotide:drug ratio of 16) in a volume of 8 mL containing 1.5 mM sodium citrate, pH 7.0, and 15 mM NaCl. After incubation for 120 min at 25°C, the reaction was stopped by ethanol precipitation. All subsequent isolation procedures were performed at 4°C. Modified plasmids were purified by 5–20% (w/v) sucrose gradient centrifugation in 25 mM Tris-HCl, pH 7.5, 1 M NaCl, and 5 mM EDTA [13]. After analysis by agarose gel electrophoresis, fractions containing covalently closed plasmids were pooled, concentrated by ethanol precipitation, redissolved in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, and dialysed overnight against 3 changes of 500 mL of the same buffer. The material was then repurified by 5–20% sucrose gradient centrifugation, followed by ethanol precipitation. The obtained drug-DNA complexes were analysed by UV spectroscopy (Fig. 2), and the amount of covalently bound CC-1065, 1 adduct/26 base pairs, was measured from the absorption at 365 nm, assuming a molar extinction coefficient of 48,000 [14]. We also constructed pUC19 containing approximately 10 CC-1065 molecules by incubating pUC19 (333 μg/mL) with 3.3 μM CC-1065 (molar nucleotide:drug ratio of 300), followed by ethanol precipitation and purification by 5–20% sucrose gradient centrifugation, as outlined above. All drug-DNA complexes were stored in small aliquots at −80°C.

Preparation of Radiolabelled DNA Fragments

The 30-mer oligonucleotide 5′-CCGGCGCCGGCCGATTACCGGCCCGGGCCC was 5′ end-labelled and purified by gel filtration, as described [12]. This oligonucleotide was annealed to the complementary strand at a molar ratio of 1:1 in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 250 mM NaCl, and the resulting double-stranded DNA fragments were stored in small aliquots at −20°C. To produce a site-specific adduct, double-stranded fragments (1.2 mg/mL) were incubated with CC-1065 (330 μM) in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, and 100 mM NaCl. After 24 hr at room temperature and in the dark, unbound CC-1065 was removed by ethanol precipitation. DNA was, then, redissolved in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, and stored at −20°C. This treatment has been shown to result in the covalent modification of the most 3′ adenine residue in the 5′-GATTA sequence located in the radiolabelled strand [15, 16]. Modification by CC-1065 was demonstrated by the formation of a short radiolabelled oli-

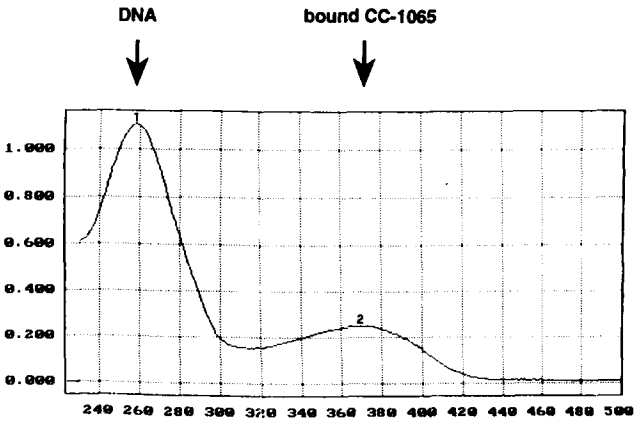

FIG. 2. Spectrophotometic analysis of pUC19 DNA modified by CC-1065. DNA (46 μg/mL) was reacted with CC-1065 (8.6 μM), and drug-DNA complexes were purified by ethanol precipitation, dialysis, and sucrose gradient centrifugation as described in Materials and Methods. The amount of DNA-bound CC-1065 (103 molecules/pUC19) was determined from its absorbance at 365 nm, assuming an absorption coefficient of 48,000 [14].

gonucleotide (presumably 5′-CCGGCGCCGGCCGATT) after incubation of the fragment at 95°C for 5 min (see below).

CC-1065 Translocation Assay

Purified drug-plasmid DNA complexes (6 μg DNA/mL) were incubated with 5′ end-labelled, but unmodified, 30-mer fragments (6 μg/mL) in 50 μL containing 10 mM Tris-HCl, pH 8.0, and 7.4 mM $MgCl_2$. After various times at 30°C, reaction mixtures were subjected to thermal treatment and analysed by denaturing gel electrophoresis as described [17, 18]. Briefly, DNA mixtures were ethanol-precipitated and redissolved in 10 μL formamide containing 0.1% Bromphenol Blue and 0.1% Xylene Cyanole as tracking dyes. To induce DNA breakage at CC-1065-DNA adducts, samples were heated to 95°C for 5 min. The resulting oligonucleotides were resolved on denaturing 20% polyacrylamide gels, which were dried, exposed to X-ray films, and quantified by scanning densitometry on a Molecular Dynamics Computing Densitometer using ImageQuant software. The appearance of radiolabelled 16-mer oligonucleotides is indicative of CC-1065 transfer from pUC19 to the 30-mer double-stranded fragment.

Denaturing Agarose Gel Electrophoresis

To demonstrate covalent modification of plasmid DNA by CC-1065, drug-DNA complexes (600 ng) were ethanol-precipitated and redissolved in 10 μL formamide. Samples were then heated to 95°C for 5 min and resolved by 1% alkaline agarose gel electrophoresis, performed in the presence of 30 mM NaOH and 1 mM EDTA [19]. After electrophoresis, the gel was washed for 45 min in 1 M Tris-HCl, pH 7.6, 1.5 M NaCl, followed by staining with ethidium bromide (0.5 μg/mL). The gel was, then, photographed under UV-transillumination, and the size distribution of the resulting DNA fragments was analysed by scanning densitometry of the photographic negatives.

RESULTS

Preparation of Characterisation of CC-1065-DNA Complexes

Defined drug-DNA complexes were prepared by reacting plasmid pUC19 with various concentrations of CC-1065. To remove unbound or weakly DNA-bound CC-1065 molecules, the reaction products were purified by ethanol precipitation and sucrose gradient centrifugation in the presence of 1 M NaCl, followed by dialysis against a low-salt buffer, and a second sucrose gradient centrifugation in the presence of 1 M NaCl (see Materials and Methods for details). After each sucrose gradient step, only the fractions containing covalently closed plasmids were collected and pooled, and fractions containing linear or nicked plasmids were discarded. The purified drug-DNA complexes were analysed by UV spectroscopy, and the amount of DNA-bound drug molecules that resisted these purification steps was measured from their absorbance at 365 nm [14]. Drug-DNA complexes containing approximately 103 CC-1065 molecules/plasmid, or 1 CC-1065/26 base pairs, were obtained by incubating DNA at a concentration of 46 μg/mL with 8.6 μM CC-1065 (Fig. 2). Complexes containing approximately 10 CC-1065 molecules/plasmid, or 1 CC-1065/269 base pairs, were obtained when 333 μg/mL pUC19 were reacted with 3.3 μM CC-1065 (data not shown).

The highest binding frequency obtained here (1 CC-1065 molecule/26 base pairs) is lower than the saturation level observed with plasmid DNA in a previous study (1 CC-1065/15 base pairs), although nearly identical initial drug-to-DNA nucleotide ratios were used [14]. This discrepancy may be explained by the more stringent purification of drug-DNA complexes performed in this study compared to the previous report [14], where these complexes were isolated by a single passage through gel filtration columns.

CC-1065 Translocation Assay

We developed the CC-1065 translocation assay to discriminate between covalent and noncovalent bonding in the purified drug-DNA complexes. As outlined in the scheme of Fig. 3A, this assay requires 30-mer DNA fragments containing a unique 5′-GATTA sequence that constitutes a preferred site for DNA alkylation by CC-1065 [15]. In these DNA fragments, only the strand containing the 5′-GATTA sequence was 5′ end-labelled before annealing to the complementary strand (Fig. 3A). Purified drug-plasmid DNA complexes were coincubated with equivalent amounts of double-stranded 30-mer fragments. After various incubation times at 30°C, DNA was recovered from the reaction mixtures by ethanol precipitation, and the transfer of CC-1065 from plasmid pUC19 to the radiolabelled 30-mer fragments was tested by heating the samples to 95°C for 5 min (Fig. 3A). This approach was based on the ability of CC-1065 to induce the release of covalently modified adenine residues accompanied by site-specific DNA strand cleavage at elevated temperatures [17], yielding shorter radiolabelled oligonucleotides of, presumably, 16 residues.

Electrophoretic analysis on denaturing polyacrylamide gels confirmed the formation of a shorter 16-mer oligonucleotide when 30-mer fragments were first modified by CC-1065 and, then, subjected to thermal treatment (Fig. 3B, lane 4). This shorter oligonucleotide was not observed when unmodified 30-mer fragments were heated to 95°C before electrophoretic analysis (Fig. 3B, lane 3). However, a significant amount of this 16-mer oligonucleotide was found when 30-mer DNA fragment were preincubated with CC-1065-modified pUC19 (103 drug molecules/plasmid) for 3 hr (Fig. 3B, lanes 11 and 12, in duplicate). This finding indicates that a considerable fraction of CC-1065 molecules was released from pUC19 and transferred to the 5′-GATTA sequence contained in the 30-mer fragments. This transfer was strictly dependent on the preincubation of

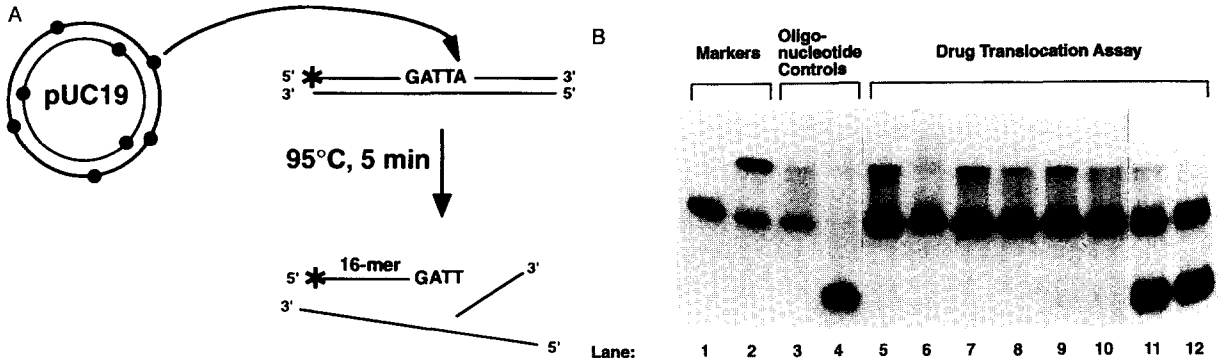

FIG. 3. Drug translocation assay. (A) Scheme illustrating the approach used to discriminate between covalent and noncovalent CC-1065-DNA binding interactions. Purified drug-pUC19 complexes (6 μg DNA/mL) were incubated with radiolabelled 30-mer fragments (6 μg/mL) containing a single 5′-GATTA preferred site for covalent modification by CC-1065. After various time periods at 30°C, reaction products were recovered by ethanol precipitation and analysed by thermal treatment (5 min at 95°C), followed by denaturing polyacrylamide gel electrophoresis. In this assay, dissociation of CC-1065 molecules from pUC19 and subsequent alkylation of the 5′-GATTA sequence is indicated by the appearance of a radiolabelled oligonucleotide of 16 residues. (B) Representative polyacrylamide gel showing efficient transfer of CC-1065 from pUC19 to the 30-mer fragment. With the exception of the samples in lanes 1 and 2, all reactions were subjected to thermal treatment before gel electrophoresis. Lane 1, size marker containing single-stranded oligonucleotides of 30 residues; lane 2, size marker containing both single- and double-stranded 30-mer fragments; lane 3, unmodified 30-mer fragment after thermal treatment; lane 4, CC-1065-modified 30-mer fragments cleaved by heat treatment to generate oligonucleotides of 16 residues; lanes 5 and 6, unmodified 30-mer fragments mixed with pUC19 (10 CC-1065/plasmid) immediately before ethanol precipitation and thermal treatment; lanes 7 and 8, unmodified 30-mer fragments preincubated with pUC19 (10 CC-1065/plasmid) for 3 hr at 30°C before thermal treatment; lanes 9 and 10, unmodified 30-mer fragments mixed with pUC19 (103 CC-1065/plasmid) immediately before ethanol precipitation and thermal treatment; lanes 11 and 12, unmodified 30-mer fragments preincubated with pUC19 (103 CC-1065/plasmid) for 3 hr at 30°C before thermal treatment. These latter incubations produced the specific thermal degradation product indicative of DNA modification by CC-1065. To optimise visualisation of the bands, the figure shows different exposure times of the same gel.

drug-plasmid DNA complexes with 30-mer fragments. In fact, the 16-mer oligonucleotide was absent when CC-1065-modified pUC19 (103 drug molecules/plasmid) and 30-mer fragments were mixed immediately prior to ethanol precipitation and thermal treatment (Fig. 3B, lanes 9 and 10), demonstrating that little or no transfer of CC-1065 occurred during the 5 min at 95°C. We also performed time-course experiments that indicated that most CC-1065 molecules translocated during the first 60 min of preincubation (data not shown). On the other hand, no transfer of CC-1065 was observed when the same experiment was performed with pUC19 containing only 10 drug molecules/plasmid, regardless of whether plasmids were (Fig. 3B, lanes 7 and 8) or were not preincubated (lanes 5 and 6) with 30-mer fragments. In fact, these reactions failed to display formation of 16-mer oligonucleotides, even when the gel was extensively overexposed during autoradiography. Thus, the translocation assay indicates that drug-DNA complexes with 103 CC-1065/plasmid contain a mixture of covalently and noncovalently bound CC-1065 moieties, and complexes with 10 CC-1065/plasmid contain only covalently bound CC-1065 adducts.

Quantitative analysis of the radioactive bands in lanes 11 and 12 of Fig. 3B revealed that nearly 80% of 30-mer fragments were cleaved after heat treatment, indicating that nearly 80% of these fragments became alkylated during the CC-1065 translocation assay. This number was highly reproducible, yielding a mean value of 3 independent experiments of 77.3 ± 2.4% CC-1065 molecules released. Because 300 ng of pUC19 (containing ~1 drug molecule/26 base pairs) were coincubated with 300 ng of 30-mer fragments (containing a single site for CC-1065 modification), we calculated that, on the average, 67.0% of CC-1065 molecules were released from their association with pUC19. Thus, these experiments demonstrate that a very large fraction of CC-1065 moieties was associated with DNA by transient, presumably noncovalent, binding forces.

Stability of Covalent Drug-DNA Complexes

CC-1065 forms inherently unstable DNA adducts that are additionally stabilised by noncovalent interactions, so that the covalent drug-DNA complex is thought to become essentially irreversible (reviewed in [1]). To confirm that only noncovalently bound CC-1065 was released during the translocation reaction, we analysed the frequency of covalent CC-1065-DNA adducts on plasmid pUC19 before and after drug translocation assays. For that purpose, we again exploited the ability of covalent CC-1065 adducts to induce strand breaks at 95°C. Drug-plasmid pUC19 complexes were tested in the translocation assay and subjected to thermal treatment, as outlined in the previous section, but the resulting degradation products were resolved by agarose gel electrophoresis under denaturing conditions. This electrophoretic technique provides detection of plasmid-derived fragments originating from CC-1065-induced

strand breaks. After visualising DNA by staining with ethidium bromide, the DNA size patterns obtained were quantified by densitometric scanning (see Materials and Methods for details).

As illustrated by the gel mobility patterns shown in Fig. 4, unmodified pUC19 subjected to thermal treatment migrated as a sharp band near the origin (Fig. 4A). Plasmid pUC19 containing an average of 10 drug molecules exhibited higher mobility, indicating the formation of short fragments by thermally induced strand breakage at sites of covalent CC-1065 modification (Fig. 4B). Plasmid pUC19, containing 103 CC-1065 molecules, demonstrated even higher gel mobility after thermal treatment, reflecting the formation of even shorter fragments as a consequence of its higher density of covalent CC-1065 modification (Fig. 4C). This increased gel mobility remained unaffected, however, when pUC19 (103 CC-1065/molecule) was preincubated for 3 hr at 30°C with an equivalent amount of 30-mer DNA fragments containing the preferred site for alkylation (Fig. 4D). Thus, the frequency of covalent CC-1065 adducts on pUC19 was not reduced during the drug translocation assay, despite the loss of nearly 70% of drug molecules from the complexes. This experiment indicated that only noncovalently bound CC-1065 molecules were released from pUC19 in the course of the translocation reaction.

This conclusion is further supported by the stability of covalent CC-1065 adducts bound to the 5′-GATTA sequence in the 30-mer fragment (Fig. 5). Radiolabelled DNA fragments were covalently modified by reaction with CC-1065, mixed with an excess of native pUC19 DNA, and analysed by thermal treatment and gel electrophoresis. In this experiment, we found the same high level of strand breakage in the modified 30-mer fragments (approximately 90%), regardless of whether the samples were heated immediately after mixing with pUC19 (lanes 3 and 4), or after co-incubating with pUC19 for 3 hr (lanes 5 and 6). This result confirms that CC-1065 adducts, after they are covalently bound to DNA, are practically irreversible. Thus,

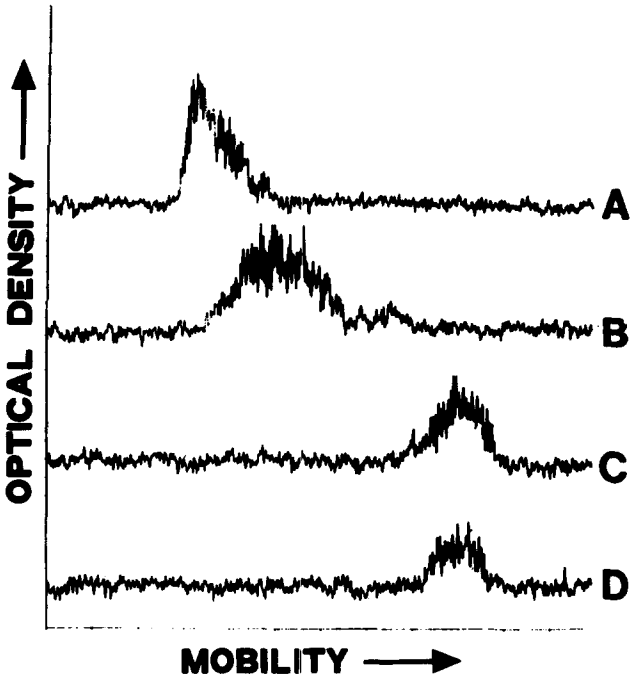

FIG. 4. CC-1065-induced strand breakage of pUC19 DNA. Plasmid pUC19 (6 μg/mL) was mixed with 30-mer fragments (6 μg/mL), ethanol-precipitated, and subjected to thermal treatment as indicated in the legend to Fig. 3, but analysed by alkaline agarose gel electrophoresis. (A) Unmodified pUC19 migrating as a sharp band near the gel origin. (B) pUC19 containing 10 CC-1065 molecules/plasmid and resulting in the formation of fragments migrating faster than intact plasmid. (C) pUC19 (103 CC-1065/plasmid) mixed with 30-mer fragments immediately prior to ethanol precipitation and thermal treatment. (D) pUC19 (103 CC-1065/plasmid) preincubated with 30-mer fragments for 3 hr at 30°C. This experiment demonstrates that the size of the obtained plasmid fragments was not increased and, hence, the frequency of covalent CC-1065 modification was not reduced during preincubation with DNA containing preferred sites for alkylation.

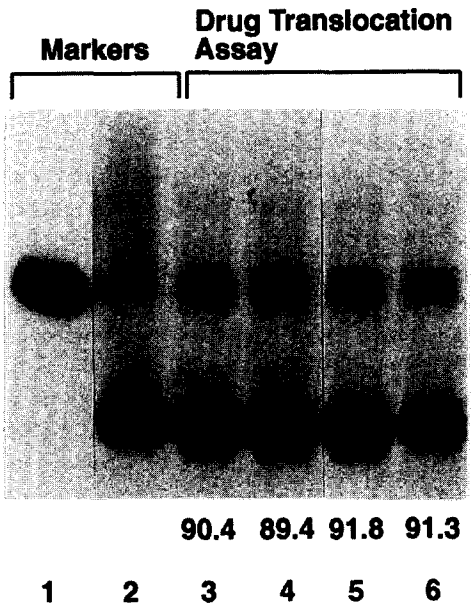

FIG. 5. Stability of covalent CC-1065 adducts. DNA fragments of 30 residues were radiolabelled and covalently modified at the 5′-GATTA site by reaction with CC-1065. These fragments (4 μg/mL) were, then, mixed with nonmodified pUC19 DNA (6 μg/mL). After ethanol precipitation, DNA was heated to 95°C for 5 min and analysed by denaturing 20% polyacrylamide gel electrophoresis as indicated in Materials and Methods. Lanes 3 and 4 (in duplicate), ethanol precipitation and thermal treatment were performed immediately after mixing the 30-mer fragments with pUC19 DNA. Lanes 5 and 6, 30-mer fragments and pUC19 DNA were coincubated for 3 hr at 30°C before ethanol precipitation and thermal treatment. The buffer conditions were identical to those used in the translocation assay of Fig. 3B. Lanes 1 and 2, markers showing the position of 30-mer and 16-mer oligonucleotides, respectively. The percentage of 16-mer oligonucleotides (nicked fragments) in each lane was determined after quantification of the radioactive bands by computer scanning densitometry.

only noncovalently bound CC-1065 was released during the translocation assay of Fig. 3.

DISCUSSION

The molecular basis for the extraordinary sequence selectivity of DNA alkylation by CC-1065 is the subject of intense scrutiny in many laboratories. The availability of different structural analogues and unnatural enantiomers of CC-1065 have greatly facilitated these studies [9–11, 20]. In particular, previous investigations revealed a primary role for noncovalent interactions between the drug molecule and the DNA minor groove in controlling its alkylation reaction and stabilising the resulting covalent adduct, although the precise contribution of these noncovalent interactions in discriminating against incorrect nucleotide sequences has not been unequivocally established [1, 9]. The present study indicates that noncovalent binding forces, in combination with the ability of noncovalently bound CC-1065 to translocate from one DNA site to another, may provide a dynamic mechanism to locate possible sequences that meet the structural requirements for DNA alkylation.

After incubation of plasmid DNA with saturating amounts of CC-1065, we obtained stable drug-DNA complexes in which nearly 70% of CC-1065 molecules remained noncovalently bound. These complexes resisted dissociation by conventional purification techniques, such as ethanol precipitation, dialysis, or sucrose gradient centrifugation (Fig. 2). Despite their tightly bound state, these noncovalent drug-DNA complexes readily dissociated when incubated with DNA fragments containing a preferred site for alkylation (Fig. 3). During this drug translocation assay, only noncovalently bound CC-1065 was removed from the complexes because the frequency of covalent plasmid modification was not decreased, even after dissociation of up to 70% of drug molecules (Fig. 4). Collectively, these results indicate that noncovalent binding forces may support a molecular translocation mechanism to locate potential alkylation sites in DNA. The first (noncovalent) step of this mechanism displays moderate sequence selectivity and involves noncovalent interactions within the minor groove, where the drug molecule senses its environment. If the local conformation is permissive for DNA alkylation, the reaction progresses with step 2 (i.e. the formation of a covalent bond with adenine). However, as demonstrated by the present study, most noncovalent CC-1065-DNA complexes are ineffective and fail to induce this alkylation reaction. In this case, the search mechanism continues by translocation to another potential alkylation site on the same, or a different, DNA molecule. The assay used in this study detects only an intermolecular mode of translocation, but this finding does not exclude the possibility that translocation may also occur intramolecularly, as has been shown for actinomycin D-DNA recognition [21]. Presumably, repeated cycles of noncovalent binding and translocation events may be necessary before all CC-1065 molecules become covalently linked to their specific alkylation sites.

A key element of this molecular translocation, or "hopping" model is the formation of transient, noncovalent, and relatively nonspecific complexes that are unable to induce DNA alkylation. These transient interactions may have escaped detection in most previous studies because consecutive noncovalent binding-translocation events at various sites in DNA may rapidly lead to efficient DNA alkylation. Zsido *et al.* [22] observed the formation of a pool of free CC-1065 in BSC-1 African green monkey cells that were exposed to CC-1065. In their study, CC-1065 was released during cell lysis, but it was not determined whether the released drug dissociated from DNA or from other, possibly nonspecific, binding sites that may occur in mammalian cells. In the present report, we have unequivocally detected noncovalent CC-1065-DNA interactions in highly purified drug-plasmid DNA complexes. Under saturating conditions yielding complexes of approximately 100 CC-1065 molecules/plasmid pUC19, the majority (~70%) of tightly bound CC-1065 molecules displayed a transient, noncovalent, and relatively nonspecific mode of interaction. These numbers indicate that plasmid pUC19 (2686 base pairs) contains approximately 30 sites for covalent CC-1065 binding (equivalent to an average of 1 covalent adduct per 90 base pairs), and approximately 70 additional sites for strong noncovalent interactions with the drug.

In summary, our results indicate that noncovalent interactions exert their essential role in targeting CC-1065 to specific alkylation sites by a binding-translocation ("hopping") mechanism. In rational drug design, the molecular components of CC-1065 responsible for this search mechanism may be adopted to enhance the selectivity of novel DNA-reactive drugs. This goal may be achieved by synthesising composite compounds, in which a particular electrophilic centre is combined with the molecular elements of CC-1065 that mediate this dynamic noncovalent binding-translocation mechanism. A similar approach has already been tested experimentally to target sulfonate esters to DNA using a minor groove-binding dipeptide [23].

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