Reaction of the Antitumor Antibiotic CC-1065 with DNA: Structure of a DNA Adduct with DNA Sequence Specificity

Abstract. *Sequence-dependent variations in DNA revealed by x-ray crystallographic studies have suggested that certain DNA-reactive drugs may react preferentially with defined sequences in DNA. Drugs that wind around the helix and reside within one of the grooves of DNA have perhaps the greatest chance of recognizing sequence-dependent features of DNA. The antitumor antibiotic CC-1065 covalently binds through N-3 of adenine and resides within the minor groove of DNA. This drug overlaps with five base pairs for which a high sequence specificity exists.*

The antitumor antibiotic CC-1065 is extremely potent (*1*) and is produced by *Streptomyces zelensis* (*2*). Our earlier studies have shown that CC-1065 binds to AT-rich sites (A, adenine; T, thymine) within the minor groove of DNA without detectable distortion of the helix and in a nonintercalative mode (*3*). The stability of the complex suggested a covalent adduct, but definitive evidence for this was lacking (*3, 4*). X-ray crystallographic data on CC-1065 reveal a molecule with a right-handed twist that mimics the pitch of B-form DNA; thus it might be accommodated within one of the grooves of DNA (*5*). As first steps toward determining the molecular basis for the potency of CC-1065, we elucidated the structure of the covalent adduct and determined the DNA sequence specificity. These results have enabled us to propose a molecular model for the CC-1065–DNA adduct in which CC-1065 binds covalently to N-3 of adenine and lies entirely within the minor groove of DNA, extending over a 5-base-pair region for which sequence specificity exists. The orientation of the DNA sequence specificity of CC-1065 in relation to the adenine covalent binding site reveals the polarity of the CC-1065 molecule in the minor groove of DNA and strongly suggests the previously unassigned absolute stereochemistry of CC-1065.

When CC-1065–DNA adducts are heated at 100°C for 30 minutes, a molecule with the CC-1065 chromophore is extractable into butanol (*6*). Up to 85 percent of the CC-1065 chromophore can be released from the DNA by this procedure, but chromatographically it differs from CC-1065. The possibility that the material solubilized by butanol was linked to a DNA base moiety was examined by using tritiated deoxyadenine, deoxyguanine, deoxycytidine, and deoxythymidine to produce, in separate experiments, labeled DNA samples modified by CC-1065. These CC-1065–(^{3}H-labeled Base)–DNA samples were heated and then extracted with butanol; the butanol contained only minor amounts of tritium in all cases except for that of the (^{3}H-labeled adenine)–DNA sample, where it contained 4.3 times more tritium than did control. Most of the tritium extracted into the butanol cochromatographed with the heat-released CC-1065 chromophore rather than with adenine. The material extracted with butanol was purified from a sample of the CC-1065–DNA adduct to obtain both ^{1}H and ^{13}C nuclear magnetic resonance spectra (Table 1). A comparison of the additional five signals found in the CC-1065–adenine adduct with those for the base moiety of 3-methyladenine showed a close correlation of chemical shift values. The downfield shifts of the resonance signals for C-4 and C-5 in the adduct were in accord with a structure resulting from opening of the cyclopropane ring (Fig. 1). Opening of the cyclopropane ring at the least substituted carbon is consistent with the structure of the ring-opened product resulting from the treatment of CC-1065 with acetic acid (*7, 8*). The alternative mode of cyclopropane ring

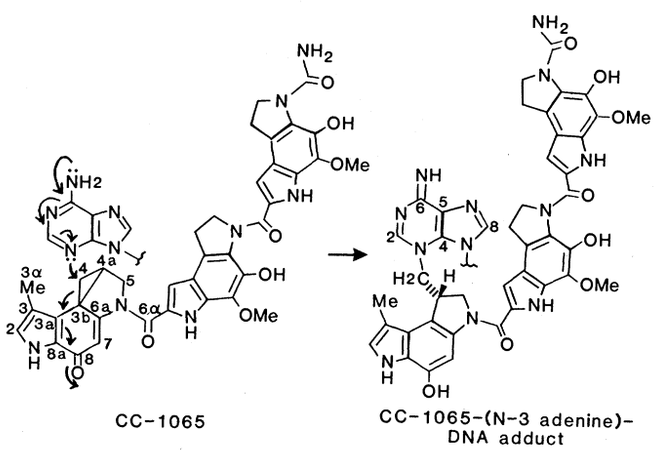

Fig. 1. Reaction of CC-1065 with DNA to form the N-3 adenine adduct.

Table 1. Partial ^{13}C NMR assignments and multiplicities for CC-1065, 3-methyladenine, and the CC-1065–N-3 adenine adduct. Spectra (500 MHz) were obtained in [$^2H_7$]dimethylformamide at 300 K on a Bruker WM-500 NMR spectrometer. Abbreviations: s, singlet; d, doublet; t, triplet; and q, quartet.

Carbon atom*	CC-1065†		3-Methyladenine		CC-1065–adenine adduct (*12*)	
	δ‡	Multiplicity§	δ	Multiplicity	δ	Multiplicity
2	124.0	d			125.0	d
3	114.1	s			112.7	s
3a	130.6	s			134.2	s
3b	||	||			110.4	s
3α	9.7	q			11.9	q
4	21.7	t			54.8	t
4a	21.8	d			40.2	d
5	50.2	t			50.3	t
6a	161.7	s			137.0	s
7	111.6	d			98.4	d
8	176.4	s			144.2	s
8a	128.6	s			125.6	s
Adenine-2			153.6	d	153.6	d
Adenine-4			150.4	s	151.5	s
Adenine-5			120.4	s	121.5	s
Adenine-6			160.4	s	155.9	s
Adenine-8			147.3	d	144.4	d

*Numbering for CC-1065 and its N-3 adenine adduct is given in Fig. 1. †Only the ^{13}C NMR data for the CC-1065 cyclopropane ring–containing subunit is shown. The chemical shift values and multiplicities for carbon atoms in the remainder of CC-1065 are virtually identical to the corresponding atoms in the CC-1065–adenine adduct. ‡Chemical shifts are relative to tetramethylsilane and referenced to the formyl carbon of [$^2H_7$]DMF (162.7 parts per million). §Multiplicity of carbon atoms was determined by insensitive nuclei enhancement by polarization transfer (INEPT) and distortionless enhancement by polarization transfer (DEPT) experiments. ||This signal was buried under a signal from the solvent but appeared at 31.5 parts per million in dimethyl sulfoxide.

opening, which would result in direct attachment of C-5 of CC-1065 to N-3 of adenine, was eliminated by consideration of the ^{13}C chemical shift value of C-5 in the CC-1065–adenine adduct. Chemical shift changes for C-3b, C-7, C-8, and C-9 in the CC-1065–N-3 adenine adduct were in accord with reduction of the indole quinone to an indole phenol. A comparison of the ^{1}H NMR spectra of CC-1065 and its adenine adduct (not shown) further substantiated the structure of the CC-1065–N-3 adenine adduct (Fig. 1).

Heating of CC-1065–DNA adducts results in breakage of the DNA strand (*3*) as well as release of a CC-1065–N-3 adenine adduct. Extensive depurination would not be expected to result in strand breakage under the neutral conditions used in these experiments (*4*), suggesting that an independent process was responsible for this event. To gain some insight into the relationship of DNA strand breakage to the covalent attachment site as well as to examine the DNA-binding sequence specificity of CC-1065, we prepared restriction enzyme fragments from SV40 (Simian sarcoma virus) DNA and T7 DNA with a single ^{32}P-labeled phosphate at the 5′ ends. A typical fragment, modified with CC-1065, was heated at 100°C for 30 minutes and analyzed on a sequencing gel (Fig. 2). Heating produced a fragment with apparent breakage at the residue adjacent to adenine on the 3′ side. Upon subsequent piperidine treatment of the previously thermally cleaved DNA fragment, the apparent break occurred about one residue to the 5′ side—that is, to the adenine residue mentioned earlier. This piperidine-dependent shift was presumably due to β elimination at an apurinic site that resulted from loss of the CC-1065–N-3 adenine adduct.

Analysis of the sequence specificity of CC-1065 within restriction enzyme fragments derived from SV40 DNA and T7 DNA showed that there are two classes of CC-1065 binding sites: 5′ PuNTTA and 5′-AAAAA (Pu, purine; N, any nucleotide base) (*9*). Both classes have an absolute requirement for adenine at one residue removed from the 5′ side of the apparent thermally induced break. Furthermore, the two classes of sequence specificities lie exclusively to the 5′ side of the covalent adenine binding site of CC-1065 and extend up to five bases. Significantly, there was no specificity for the base at the 3′ side of the adenine residue at which the thermally induced break occurred. One possibility for this unusual cleavage pattern is that a direct interaction between CC-1065 and the deoxyribose phosphate backbone may occur, resulting in strand breakage. Analysis of the opposite strand around CC-1065 binding sites did not show any residual modification of the DNA after drug binding (*9*).

Stereo drawings of the CC-1065–(N-3 adenine)–DNA adduct were prepared (Fig. 3) on the basis of the known position of covalent attachment and an orientation wherein the CC-1065 overlaps with the specified sequence of base pairs. The drawings show a remarkably snug fit of the CC-1065 in the minor groove, in which it is apparent that only certain base pairings can be accommodated by CC-1065. While the relative stereochemistry of the cyclopropane ring has been determined (*5*), the absolute stereochemistry has not. The stereo drawings show that the CC-1065–N-3 adenine adduct can only be accommodated if the absolute configuration at C-5 is as shown in Fig. 1.

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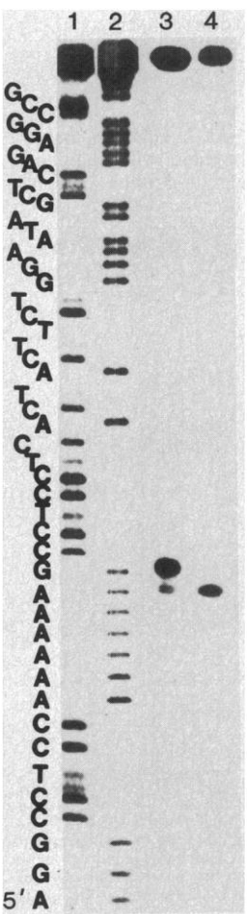

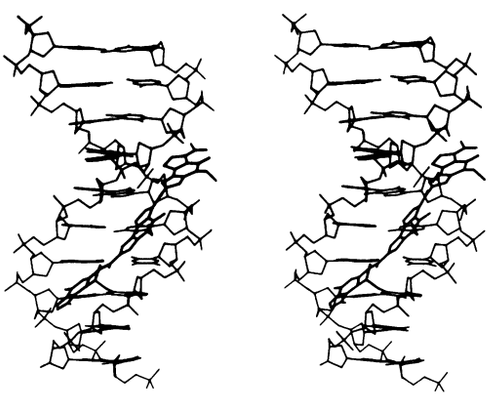

Fig. 2. The CC-1065 binding site within a region of the SV40 early promoter. A DNA restriction fragment from the SV40 genome [5172 to 5241; marked with a single ^{32}P-labeled phosphate at the 5′ end at 5172; numbered as described (*10*)] was prepared by means of established procedures (*11*) and was subjected either to sequencing reactions or to treatments with CC-1065. (Lanes 1 and 2) Maxam-Gilbert C+T and A+G reactions, respectively (C, cytosine; G, guanine). (Lane 3) DNA was resuspended in 20 μl of 7.5 m*M* NaCl and 0.75 m*M* sodium citrate (*p*H 7.4) containing 2.8 pmol of CC-1065 and was incubated for 24 hours at 4°C. After removal of unbound drug by precipitation of the DNA with ethanol, the DNA was resuspended in 0.1 ml of 15 m*M* NaCl and 1.5 mM sodium citrate (*p*H 7.4) and heated at 100°C for 30 minutes in a sealed test tube. The DNA was then precipitated with ethanol and dried under vacuum. (Lane 4) Identical to lane 3 except that, after removal of unbound drug by precipitation of the DNA with ethanol, the DNA was subjected to heat treatment with piperidine (*11*). After sequencing reactions or CC-1065 treatments, samples were subjected to electrophoresis and analyzed by autoradiography as described (*11*). Fig. 3. Stereo drawings of the CC-1065–DNA adduct.

References and Notes

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