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REACTION OF CC-1065 AND SELECT SYNTHETIC ANALOGS WITH DNA

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The molecular mechanisms for sequence specific recognition of DNA by small molecules and proteins has received considerable attention in the last few years. The primary mechanism for DNA sequence recognition of minor groove binding sites by small molecules such as the netropsin/distamycin group is proposed to occur by close van der Waals contacts (2), although more recent work has demonstrated that there may also be other as yet unidentified factors to consider (2). While a considerable effort has gone into determining the non-covalent interactions that lead to sequence specific recognition of DNA, (see Ref. 3, 4 for reviews) much less is known about the origin of sequence specificity of covalent DNA modification.

CC-1065 is an antitumor-antibiotic produced by Streptomyces zelensis (see Ref. 5, 6 and for reviews 7,8). It is active against several experimental murine tumors in vitro and is 100 times more potent than Adriamycin against a broad spectrum of tumors in the cloning assay (9). Unfortunately, CC-1065 produces a delayed death in mice (10) which will preclude its clinical application, although synthetic analogs are now available which show more promising chemotherapeutic properties (11,12). CC-1065 binds covalently through N^3 of adenine and lies snugly within the minor groove of DNA, covering a four base pair region to the 5' side of the covalently modified adenine (13). Upon thermal treatment of CC-1065-(N^3-adenine)-DNA adducts, cleavage of the N-glycosidic linkage and subsequent backbone breakage occurs to the 3' side of the covalently modified adenine to leave a 5'-phosphate on the 3' side of the break and a tattered deoxyribose on the 5' side (14) (figure 1). Using this strand breakage assay, we have determined that the binding

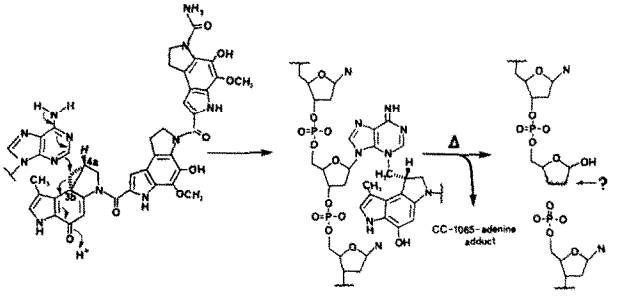

Figure 1 Reaction of (+)-CC-1065 with N^3 of adenine in DNA and Products from Thermal Cleavage Reaction (10,11). The exact nature of the species generated on the 5' side of the strand break is unknown.

sites for CC-165 belong to two subsets; 5'AAAAA* and 5'PuNTTA*, where * indicates the covalently modified adenine (14). The construction of a site-directed CC-1065-(N^3-adenine)-DNA adduct in a 117 base-pair fragment (15) has allowed us to determine the effect of drug binding on local DNA structure. DNase I footprinting and restriction enzyme analysis demonstrates that CC-1065 has an asymmetric effect on DNA structure which extends more than one helix turn to the 5' side of the covalent binding site (16).

In this study the DNA binding, sequence specificity and biological potency of CC-1065 and a select group of

synthetic analogs has been evaluated. While the major function of the non-alkylating subunits of CC-1065 is to increase the binding affinity of the drug molecule for the minor groove of DNA, the alkylation subunit alone is sufficient to mediate the DNA sequence specificity of the entire drug molecule. Furthermore, covalent binding of the alkylating subunit of CC-1065 to DNA produces the same large asymmetric effect on DNA structure as the entire drug molecule. These results suggest that the molecular basis for the DNA sequence specificity of CC-1065 is related to the asymmetric effect on local DNA structure. Therefore, we conclude that CC-1065 binds selectively to adenine containing sequences which have an increased propensity to undergo the requisite conformation change in DNA structure required to accommodate covalent binding. These experiments provide the first experimental date suggesting the prime importance of the covalent binding reaction in determining the sequence specificity of a DNA binding ligand.

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