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STRUCTURE OF DNA DAMAGED BY UV AND PSORALEN

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Psoralens are a class of naturally occuring and synthetic furocoumarins (for a recent review see 1) that are used for treatment of psoriasis and other skin disorders (2). In addition, psoralens have been used to inactivate viruses (3), and can cause mutagenesis (for a review see 4). They have also been used as *in vitro* and *in vivo* probes of nucleic acid structure and function because of their ability to covalently link nucleic acids by forming cross-links between opposing strands of duplex regions of DNA (5, 6, 7, 8). A three-step mechanism of psoralen cross-linking with DNA has been proposed (9). The planar psoralen first intercalates into a double helical region, and initial UV irradiation (320-400 nm) induces a single cyclobutane addition with a pyrimidine base. The bifunctional nature of psoralens allows the initial photochemistry to occur on either the furan or pyrone side. Only the furan-side monoadduct still absorbs in this wavelength region and a second photoaddition can then take place on the pyrone side with another pyrimidine forming the diadduct or cross-link. Reaction products of psoralen with random sequence DNA indicate that thymines are the preferred site for monoadduct formation (10). Psoralens also show a clear preference for cross-linking at 5' TpA 3' sites in DNA (11, 12). The stereochemistry of the addition product between the 4',5'(furan) or 3,4(pyrone) double bonds of psoralen and the 5,6 double bond of pyrimidine bases has been determined to be the cis-syn conformation (10, 13). This has been confirmed by the three-dimensional structure of the monoadduct determined by X-ray crystallographic methods (14). Construction of physical models using this monoadduct structure led to the proposal that psoralen cross-links caused a sharp kink in the double helical DNA structure and unwinding of the duplex (15). A detailed molecular model of psoralen cross-linked DNA, using the monoadduct structure as the basis for computer model building and energy minimization calculations, has been presented (16).

We have recently used NMR methods to determine a three-dimensional model of an 8 base-pair DNA fragment cross-linked with psoralen. The duplex form of the self-complementary deoxyribonucleotide, d-GGGTACCC, contains a psoralen cross-linkable site at the center of the duplex. The cross-link was formed by UV irradiation of a mixture of the purified DNA octamer and 4'-(aminomethyl)-4,5',8-trimethylpsoralen (AMT). Structural information was obtained using both one and two-dimensional NMR techniques. Two-dimensional NOE experiments were used to assign the spectrum and estimate distances for many pairs of protons in the cross-linked DNA. These inter-proton distances have been used to develop a model for the structure of this molecule in solution. Structural parameters obtained are qualitatively consistent with a previously proposed model for kinked and unwound cross-linked B-form DNA derived from crystallography and molecular modeling(16). The NMR derived model has a 53 degree bend into the major groove occuring primarily at the site of drug addition, and a 56 degree unwinding spanning the 8 base pair duplex. Schematic representation of (a) unmodified double stranded DNA, (b) psoralen cross-linked double stranded DNA, with indications of the directions of unwinding,are shown below.

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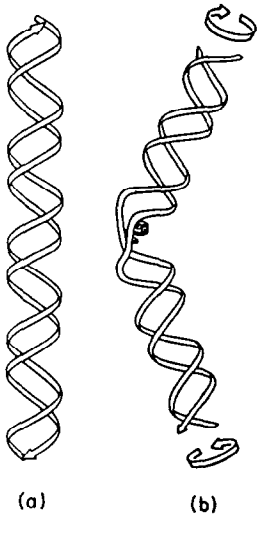

(a) (b)

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