Sharp Kink of DNA at Psoralen-cross-link Site Deduced from Crystal Structure of Psoralen-Thymine Monoadduct


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Psoralens are a class of tricyclic aromatic heterocycles that have been used as chemical probes to study DNA and RNA structures (for review, see Hearst 1981). On exposure to UV light, psoralens form chemical cross-links to the DNA bases in three steps (Isaacs et al. 1977; Johnston et al. 1977). The first step is intercalation of psoralen between two adjacent base pairs. The second step is formation of a monoadduct, i.e., one psoralen photoreacts to one strand of DNA. The third step is the cross-linking of the same psoralen to the other strand of DNA.

Light-induced cross-linking of double-stranded nucleic acids by psoralens has been exploited to locate, in vivo or in vitro, those double-helical regions of DNA or RNA that can accommodate any structural changes caused by the psoralen cross-links. To determine three-dimensional structural parameters of the cross-link, we have solved the crystal structure of the psoralen-thymine monoadduct formed in photoreaction between calf thymus DNA and 8-methoxypsoralen (8MOP).

Figure 1 shows the chemical structures and numbering system used for 8MOP and thymine. The photoaddition occurs at the double bonds between C12 and C13 of the furan ring (site I) and between C3 and C4 of the pyrone ring (site II). The preferred target is the double bond at TC5 and TC6 of thymine, forming cyclobutane rings by reacting at either site I or site II of the psoralen.

There are eight possible configurations for psoralen-thymine monoadducts (Fig. 2) and 64 for diadducts. Spectroscopic and other studies have narrowed this down to a few possible configurations, and recent nuclear magnetic resonance (NMR) studies on psoralen monoadducts (Straub et al. 1981; Kanne et al. 1982b) and crystallographic studies described here prove unambiguously that the biologically relevant structures have the "cis-syn" configuration. Such drastic reduction in the number of configurations comes from the fact that the double-helical DNA conformation imposes very stringent restrictions on modes of psoralen interaction. Thus, presumably, only a particular configuration can be formed.

We describe here the structural details of a psoralen-thymine monoadduct obtained in a biological environment and the consequences of the photo-cross-link between 8MOP and double-helical DNA.

**EXPERIMENTAL PROCEDURES**

The monoadduct of 8-methoxypsoralen-thymine (8MOP-T) was synthesized and purified as described below (Kanne et al. 1982b). 8MOP was added to DNA and then irradiated with UV light at 320–380 nm. The psoralen-DNA solution was extracted with chloroform to remove unreacted psoralen and its photodegradation products. This DNA-psoralen photoprodct was precip-

![Figure 1. Numbering system for 8MOP and thymine used in this paper.](image1)

![Figure 2. Nomenclature and schematic representation of the eight possible configurational isomers for 8MOP-thymine monoadduct.](image2)
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RESULTS AND CONCLUSIONS

Psoralen Photoreacts to Both A-T and T-A Sequences in DNA

In the unit cell there are three 8MOP-T molecules of one-handedness, and the other three have other-handedness. This suggests that 8MOP can form photomonoadducts to an A-T sequence as well as T-A sequences in double-helical DNA (see Fig. 4). Although two isomers are present in a 1:1 ratio in crystals, isolation results suggest that the ratio may be 3:2 in the hydrolysate (Kanne et al. 1982b).

Angle between Planes of Psoralen and Base Is Variable

The difference among the three unique molecules in the unit cell is best manifested by the interplanar angle between the psoralen and thymine moieties, as shown in Figure 5. The interplanar angle ranges between 44.11°
Figure 6. Schematic drawing of double-helical B-DNA (cylinders) linked by psoralen photo-cross-links. The T-A base pairs on both sides of psoralen moiety are shown as rectangular plates. This is based on a detailed molecular model built using skeletal atomic components.

and 53.45°. This suggests that the interplanar angle is not fixed but has a limited range of flexibility, with its values depending on the environment. This, in turn, implies that psoralen-cross-linked DNA can still have limited flexibility at the site of the cross-link.

Cis-Syn Configuration on Both Sides of Psoralen Plane

The configuration found in this crystal structure (cis-syn) allows one to build molecular models for an 8MOP-T2 diadduct, where another thymine forms a photoadduct at the pyrone ring. Our model building shows that for each 8MOP-T monoadduct enantiomer, there is only one 8MOP-T2 diadduct that can be built and accommodated in double-helical DNA. This unique diadduct had the cis-syn configuration at the pyrone ring, as shown in Figure 4. This is consistent with the observation by Kanne et al. (1982a).

Psoralen Cross-linking Introduces a Sharp Kink in DNA Structure

As pointed out earlier, the angle between the thymine and psoralen moieties in the structure ranges from 44° to 53° when the cyclobutane ring is formed at the furan ring. If one assumes similar geometry at the pyrone ring, then one can easily see that the extent of distortion to the DNA that would be induced by psoralen cross-linking is substantial. Molecular model building based on this structure suggests that the DNA may kink as much as 70°. Psoralen cross-linking will not only unwind DNA, as was previously shown by the superhelical density studies (Weisehahn and Hearst 1978), but also introduce a very sharp kink at the site where psoralen is introduced (Figs. 6, 7, and 8). This kind of distortion is important in that any portion of duplex DNA that cannot accommodate psoralen intercalation or a sharp kink will not form psoralen cross-links. This line of reasoning leads one to conclude that the psoralen-DNA photo-cross-linking may occur only in those regions of duplex DNA that are flexible and are not constrained by proteins or a three-dimensional structure (Hanson et al. 1976).

The sharp kink of the model suggests that, after the monoadduct formation, there must be a drastic conformational change to form a kink before the second photoreaction can occur to complete the cross-linking. The necessity for the kink provides a possible explanation for the observed relaxation time of 1 µsec between the monoadduct and formation of the diadduct (Johnston et al. 1981). This is shown schematically in Figure 7.

Recently, Land et al. (1982) published a short communication reporting the structure of a photoadduct obtained by UV irradiation of a mixture of 8MOP and thymine in an ice-methanol matrix in the presence of benzophenone. Although this adduct was made under nonbiological conditions without the structural constraints of the double-helical DNA and was presumably one of many products formed in the reaction, the structure they obtained is the same as that reported here.

Figure 7. Presumed steps in psoralen-DNA cross-linking.
Figure 8. Molecular model of 8MOP-DNA cross-link.
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