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Structural determination of the ultraviolet light-induced thymine-cytosine pyrimidine-pyrimidone (6-4) photoproduct

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ABSTRACT

Ultraviolet light induces damage to DNA, with the majority of the damage expressed as the formation of cyclobutane dimers and pyrimidine-pyrimidone (6-4) photoproducts. The (6-4) photoproducts have been implicated as important UV light-induced premutagenic DNA lesions. The most abundant of the (6-4) products is the thymine-cytosine pyrimidine-pyrimidone (6-4) photoproduct, or TC (6-4) product. The structure of the TC (6-4) product was deduced by proton NMR, IR, and fast atom bombardment mass spectroscopy, and the product was found to differ from the previously described photoadduct, Thy(6-4)Pyo, by the presence of an amino group at the 5 position of the 5' pyrimidine. The implications of this structure on DNA base pairing and the induction of ultraviolet light-induced mutations are discussed.

INTRODUCTION

Ultraviolet light exposure to cells results in lethality and the induction of mutations (1,2). The ultimate target of UV light exposure is the cellular DNA, resulting in the formation of DNA photoproducts (3). The major UV light-induced DNA photoproduct is the cyclobutane dimer formed between adjacent pyrimidines in the DNA (4,5). Recent evidence suggests that another class of UV light-induced photoproducts, the pyrimidine-pyrimidone (6-4) photoproducts, are major DNA premutagenic lesions, at least in prokaryotes (6-10). The (6-4) products are precursor compounds to a series of bipyrimidine photoproducts first described by Wang and his colleagues (11-16) that were isolated from DNA following acid-hydrolysis treatment. The most abundant of the Wang compounds, Thy(6-4)Pyo, was characterized extensively and found to be derived from thymine and cytosine (13,14). It was later shown that the TC (6-4) product is the precursor photoproduct to Thy(6-4)Pyo that is formed in DNA directly by UV irradiation (17).

The (6-4) products can be readily identified in UV irradiated DNA because they possess several unique properties. The photoproducts are fluorescent, and have red-shifted UV absorbance spectra (17). The (6-4) products are also

alkaline labile (17,18), and are formed with about one-tenth the frequency of cyclobutane pyrimidine dimers (6,18). With the exception of the TT (6-4) product (15), the molecular structure of the (6-4) products have not been established directly. It has been suggested previously that the TC (6-4) product differs from Thy(6-4)Pyo by the presence of an amino group at the 5 position of the 5' thymine (12,13). However, this evidence is based on an analogy with the pathway for formation of the TT (6-4) product, which is thought to occur via a short-lived oxetane ring intermediate (12,15); in contrast, the TC (6-4) product is thought to form via an azetidine ring intermediate (11,12).

In this study, the structure of the TC (6-4) product is deduced based on evidence obtained by infrared, nuclear magnetic resonance, and mass spectroscopy. It was found that the structure of the TC (6-4) product was that previously suggested to occur in DNA by Wang and his colleagues, and confirms that the product Thy(6-4)Pyo results from the deamination of the TC (6-4) product.

MATERIALS AND METHODS

Preparation of the TC (6-4) Product

The dinucleotide 2'-deoxythymidylyl-(3' -> 5')-2'-deoxycytidine (dTpdC, Sigma Chemical) was suspended in water at 1 mg/ml and 10 ml of the dinucleotide was irradiated on ice in 20 ul drops. The compound was exposed to a total dose of 10^5 J/m^2 of primarily 254 nm UV light from a GE15T8 germicidal lamp. Following UV light exposure, the compound was lyophilized and resuspended in 250 ul of water. The TC (6-4) product was isolated by reverse phase HPLC as previously described (17). A yield of 800 ug of purified TC (6-4) product was obtained from 10 mg of starting material.

Proton Nuclear Magnetic Resonance

The TC (6-4) product (500 ug) was lyophilized twice in the presence of deuterium oxide (Stohler Isotopes, 99.96% D)and was suspended in 300 ul of D_2O. Proton NMR was performed on a Nicolet 360 MHz FT-NMR. The sample received a 90° pulse, and the pulse recycle time was 0.68 sec.

Infrared Spectroscopy

The IR spectrum of TC (6-4) product was obtained on a Perkin-Elmer Model 781 infrared spectrophotometer.

Mass Spectroscopy

The TC (6-4) product (400 ug) was dissolved in 1:1 water/glycerol (total volume, 6 ul). The Fast Atom Bombardment (FAB) mass spectrum was recorded in

both the positive and negative ion modes using a Varian MAT 732 mass spectrometer and xenon as the neutral beam. Modifications for FAB and operating conditions for this instrument have been described (19).

RESULTS

The TC (6-4) product was prepared in dinucleotide form by direct irradiation of the compound dTpdC with 10^5 J/m^2 of 254 nm UV light. The product was purified by reverse phase HPLC, and from a total of 10 mg starting material, a yield of 800 ug was obtained. The UV absorbance spectra of the purified compound was the same as that reported previously (17), and the molar extinction coefficient at neutral pH of this compound is 2.06 x 10^3 at 310 nm. The proposed structure of the compound is given in figure 1.

The infrared spectra of the TC (6-4) product is similar to that of Thy(6-4)Pyo (13). A strong band at 9.3 u was noted that results from an asymmetric stretching vibration of the phosphate group (data not shown), and from C-O stretching vibrations in the deoxyribose moieties (20). A band at 8.2 u was seen, resulting from antisymmetric stretching vibrations of the phosphate group.

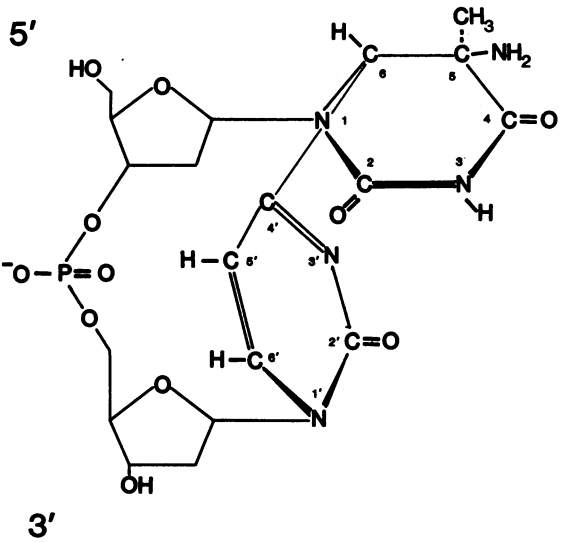

Figure 1. Proposed molecular structure of the dinucleotide form of the TC (6-4) product.

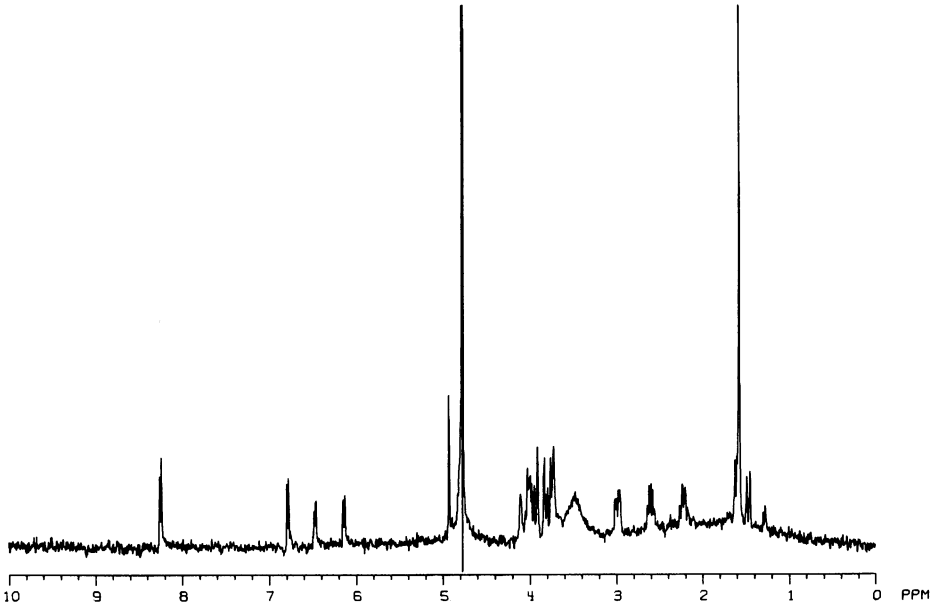

Figure 2. Proton NMR spectrum (360 MHz) of the TC (6-4) Product in D_2O. The chemical shifts are relative to TSP.

The 360 MHz proton NMR spectra of the TC (6-4) product in D_2O is shown in figure 2. A feature of the spectrum noticed initially is the presence of a broad band at δ 3.5, which is indicative of amino group protons that undergo rapid exchange with the deuterium molecules present in the sample. This amino group is assumed to be lost upon acid hydrolysis treatment to form the compound Thy(6-4)Pyo, and this resonance is absent in the proton NMR spectra of Thy(6-4)Pyo (13).

In figure 3, the NMR spectra downfield from the HOD resonance is shown. By comparison of the proton NMR spectra of Thy(6-4)Pyo (13) and the TT (6-4) product (15), assignments of the proton residues are possible. The doublet at δ 8.2 is due to the proton at the C6' position and the doublet at o 6.7 is due to the proton at the C5' position in the group -CH=CH- . The singlet at δ 4.9 is assigned to the proton on C6, based on the position of the analogous proton seen in the proton NMR spectra in D_2O of the TT (6-4) product. The doublets at δ 6.1 and δ 6.4 are assumed to arise from protons on the deoxyribose ring (21,22).

In figure 4, the upfield portion of the NMR spectra is shown. The large singlet at δ 1.6 results from the CH_3- group at position C5, and the

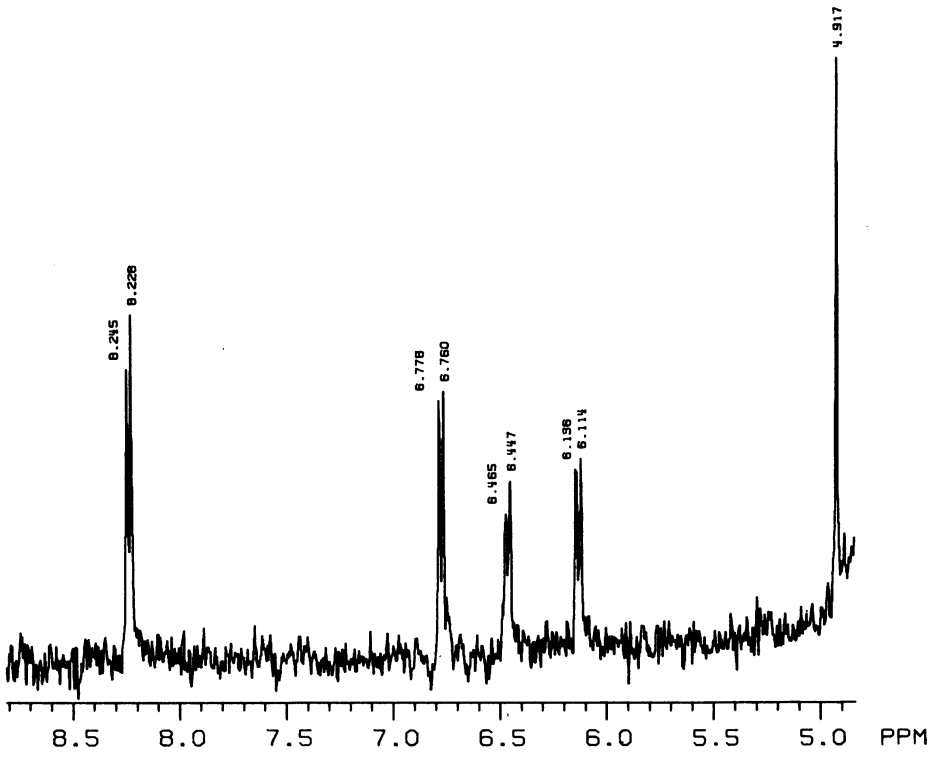

Figure 3. The downfield portion of the proton NMR spectrum from 4.8 to 8.5 ppm of the TC (6-4) product.

presence of this singlet has been seen in the proton NMR spectra of the TT (6-4) product and Thy(6-4)Pyo (13,15). As described above, a broad band is also seen at δ 3.5 resulting from the amino group at position C5. The other resonances result from protons present on the deoxyribose rings and show similar spectral patterns to that described for dTpdC, dTpdT, and the cyclobutane dimers of dTpdT and dTpdC (21,22).

The fast atom bombardment (FAB) mass spectra of the TC (6-4) product was obtained in both the positive and negative ion modes. The negative ion FAB mass spectra for the spectral region from m/z 450-700 is shown in figure 5. A peak corresponding to the anion of the TC (6-4) product is seen at m/z 530, its glycerol adduct is seen at m/z 622 and a fragment is seen at m/z 592 that arises by loss of formalin from the m/z 622 glycerol adduct. The ions at m/z 459, 551, and 643 result from matrix clusters of glycerol.

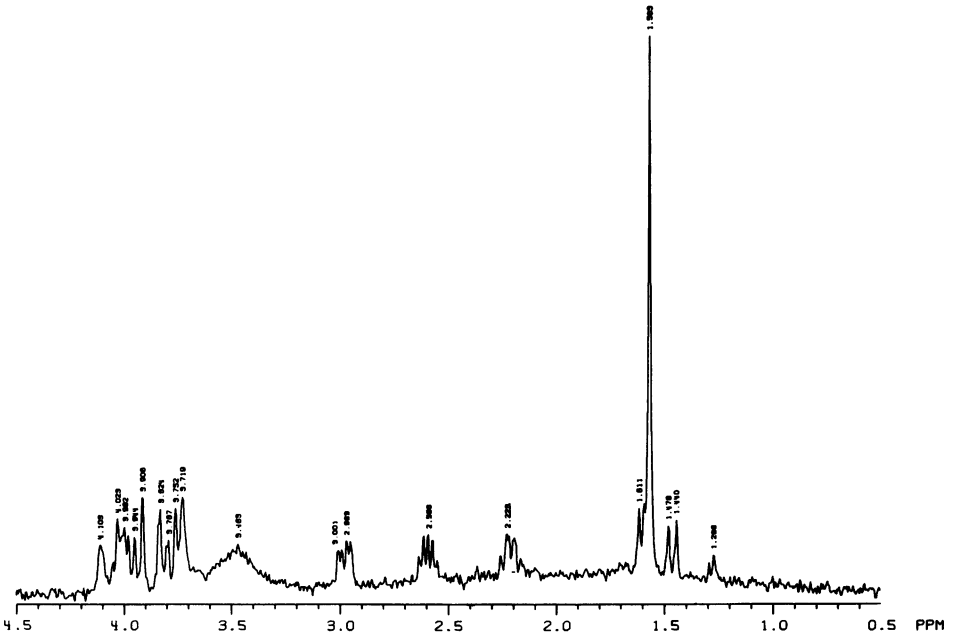

Figure 4. The upfield portion of the proton NMR spectrum from 0.5 to 4.5 ppm of the TC (6-4) product.

The exact mass of the compound was measured in the positive ion mode $(anion + 2H)^+$, and an ion was observed at m/z 532.1429. When the exact mass was calculated for $C_{19}H_{27}N_5O_{11}P$, a value of m/z 532.1445 was obtained. It is concluded that the structure shown in figure 2 is that of the TC (6-4) product based on the exact mass, NMR, and IR spectra.

DISCUSSION

The evidence presented here confirms the structure of the TC (6-4) product as that previously predicted to be found in UV irradiated DNA by Wang and his colleagues (12,13). This structure is found in DNA following the spontaneous opening of a short-lived four-membered azetidine ring structure formed between an adjacent thymine and cytosine following UV irradiation. A X-ray crystallographic study of the TT (6-4) product has demonstrated that the planes of the pyrimidine and pyrimidone rings of the photoproduct are separated by an angle of 96° (23,24). Based on an examination of three-dimensional models of the TC (6-4) product, a similar angle of ring separation would be predicted.

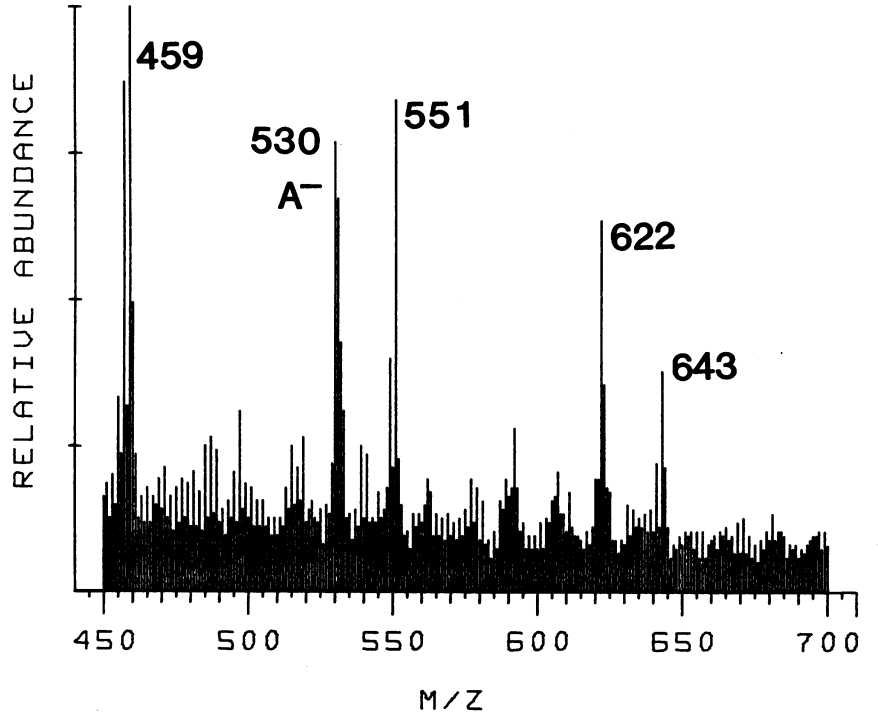

Figure 5. The negative ion FAB mass spectrum of the TC (6-4) product form m/z 450 to m/z 700. The major ion (A^-) corresponding to the TC (6-4) product is found at m/z 530.

What are the implications of the TC (6-4) product structure in base pairing? An examination of models of DNA containing the TC (6-4) product suggests that the 5' pyrimidine ring of the compound may stille be able to Watson-Crick base pair with the opposite adenine on the adjacent strand. The 3' pyrimidone ring can no longer base pair with opposite guanine as the amino group has been transferred to the 5' ring and the angle of the plane of the ring would not permit Watson-Crick base pairing in the B form of DNA. This structure causes a distortion of the phosphodiester backbone, and such distortion may contribute to the recognition of (6-4) products by the uvrABC endonuclease complex of E. coli (25,26).

Recent evidence suggests that the (6-4) products are premutagenic lesions in E. coli (6-10). Most of the UV light-induced mutations found at sites of (6-4) product formation are transitions that are mapped to the 3' side of a dipyrimidine sequence (6-8). The structure of the TC (6-4) product suggests a

possible mechanism for the induction of mutations at (6-4) product sites. The 3' pyrimidone ring of the compound can no longer base pair to the adjacent strand of DNA, and the angle of the plane of this ring is such to leave a gap in the DNA strand, such as would be seen for an apyrimidinic (AP) site. It has been shown previously that E. coli polymerase I preferentially inserts adenine residues at AP sites (27). The insertion of an adenine opposite the 3' pyrimidone ring of the TC (6-4) product could explain the large number of C -> T transitions that have been observed at sites of formation of this photoproduct.

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