CHARACTERIZATION OF THE PHOTODIMERS FROM DNA

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Received April 3, 1967

The thymine dimer ($\widehat{TT}$), isolated from ultraviolet (UV) irradiated DNA by acid hydrolysis, represents only one of the possible four isomeric species (Weinblum and Johns, 1966). If this dimer is formed in a single strand of DNA between adjacent thymine molecules, and if we assume the Watson and Crick structure for DNA, then the dimer should have the structure given in Fig. 1.

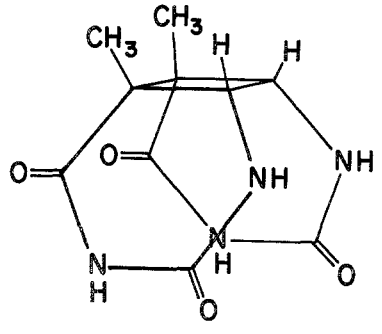

Fig. 1. Proposed structure of Thymine Dimer from UV irradiated DNA.

There is good evidence that the dimer produced by UV irradiation of a frozen thymine solution is the same as that produced in DNA (Weinblum and Johns, 1966; Blackburn and Davies, 1966 a) with the structure shown in Fig. 1 (Wang, 1963; Blackburn and Davies, 1965, 1966 b; Weinblum et al., 1966). However, an unequivocal proof of their identity has not yet been reported.

Here we will present infra red (IR) spectroscopic data which proves that the dimers from DNA and from frozen thymine solution are identical. We will

also show, by the same method, that the mixed thymine-uracil dimer ($\widehat{TU}$) from UV irradiated DNA (Setlow and Carrier, 1966) is the same as that produced by the irradiation of a mixed frozen thymine-uracil solution. The $\widehat{TU}$ and also a uracil dimer ($\widehat{UU}$) obtained from DNA (Dellweg and Wacker, 1962) are formed respectively from thymine-cytosine dimer ($\widehat{TC}$) and cytosine-cytosine dimer by deamination.

MATERIALS AND METHODS

1. Isolation of Dimers from DNA

a) 1.5 g of salmon sperm DNA (Cal. Biochem. Corp., Los Angeles, Cal.) were dissolved in 1.5 l water. The solution was irradiated in a 2 mm layer with germicidal lamps (General Electric, Catalogue No. G30T8) at a distance of 7 cm for one hour. This exposure caused a 3% loss of the original absorbance at 260 mμ. The solution was then evaporated to dryness. The residue was hydrolysed with 3 ml of 70% perchloric acid at 100°C for 45 min. Then 150 ml of water were added and the solution was neutralised with 10N KOH and left overnight. The $KClO_4$ precipitate was filtered off.

b) The filtrate was brought to pH 10 with ammonium hydroxide and applied to a 4.5 x 20 cm Dowex 1 x 8 column in the formate form. Elution was performed with a linear ammonium formate gradient. The mixing chamber contained 2 ml of formic acid, 10 ml of conc. ammonium hydroxide and 2 l of water, while the reservoir contained 5 ml of formic acid, 13 ml of conc. ammonium-hydroxide and 2 l of water. Although these conditions were sufficient to separate isomeric thymine dimers (Weinblum and Johns, 1966) only one dimer-containing peak was observed. Dimers were detected by reirradiating aliquots of the column fractions at 254 mμ and observing recoverable absorbance at 264 mμ (see Weinblum and Johns, 1966). The dimer containing peak eluted between 300 and 600 ml of effluent. The material contained in the peak was subjected to paper chromatography which showed that it contained cytosine as well as dimers. (Whatman 3MM paper; solvent: n-butanol/water 86/14 v/v; Rf values: cytosine 0.24, dimer 0.11).

To elute thymine, adenine, and guanine from the column, an additional stronger elution gradient was added (5 ml of formic acid, 13 ml of conc. ammonium hydroxide and 2 l of water in the mixing chamber and 15 ml of formic acid, 15 ml of conc. ammonium hydroxide and 2 l of water in the reservoir).

c) The effluent containing the dimer peak was evaporated to dryness to remove the ammonium hydroxide, then re-dissolved in water and put on a short Dowex 50 column in the H^+ form to remove ammonium ions and cytosine. The effluent was flash evaporated to dryness to remove formic acid.

d) The residue was dissolved in water. The pH was adjusted to 10 with ammonium hydroxide and then applied to a second 2 x 40 cm Dowex 1 x 8 column in the formate form. The gradient was produced by a mixing chamber containing 0.5 ml of formic acid, 5 ml of conc. ammonium hydroxide and 1 l of water, while the reservoir contained 2 ml of formic acid, 6.5 ml of conc. ammonium hydroxide and 1 l of water. The elution profile consisted of two main peaks and a very small one (Fig. 2). The peak samples were desalted as under (c).

e) The residues were recrystallized twice from water, the precipitates were collected by centrifuging at 10,000 g for 10 min. at $4^{\circ}C$, washed with cold water and alcohol and dried in a vacuum dessicator. The total yield of dimers from 3 g of irradiated DNA was 3 mg of dimer from peak I and 0.7 mg of dimer from peak II.

2. Isolation of Dimers from Frozen Solutions

a) A mixture of $\widehat{TT}$, $\widehat{TU}$ and $\widehat{UU}$ was prepared by irradiating a frozen aqueous solution $10^{-3}M$ in thymine and uracil (Wacker et al., 1961). The irradiated material was recrystallized once from water and about 30 mg of this mixture was separated on the column described under (1d) to give three peaks (See Fig. 2). The peak samples were treated as described under (1c) and (1e).

b) About 0.1 mg of each sample was dissolved in water, reirradiated for 5 min. with a germicidal lamp and then spotted on chromatographic paper (Whatman 3 MM; solvent: n-butanol/water 86/14 v/v). The sample from the first peak produced only thymine on the chromatogram, that from the second yielded

thymine and uracil in the ratio of 1:1, while that from the third peak gave only uracil (Rf values: thymine 0.50, uracil 0.35).

3. Infra Red Spectra

To obtain IR spectra, KBr pellets were pressed from 0.4 mg dimer and 200 mg KBr and spectra were recorded with a Perkin Elmer IR Spectrophotometer Model 237B.

RESULTS AND DISCUSSION

The acid hydrolysate of UV-irradiated DNA, separated on the first column (Materials and Methods 1b) showed four peaks corresponding to the four DNA bases, and two additional peaks. One of them, containing dimers, overlapped the cytosine peak. The second one eluted between the thymine and adenine peaks in the stronger elution gradient at about 0.11 N ammonium formate. This peak showed maximum absorbance at 315 mμ and is probably related to the photoproduct described by Pearson et al. (1965).

It was assumed that the dimer peak contained $\widehat{TT}$ and $\widehat{TU}$. In an effort to get these products separated, a longer ion exchange column was used. In order to test its resolution, a mixture of $\widehat{TT}$, $\widehat{TU}$ and $\widehat{UU}$ was applied to this column and 3 dimer peaks were obtained. After conversion to monomers by re-irradiation

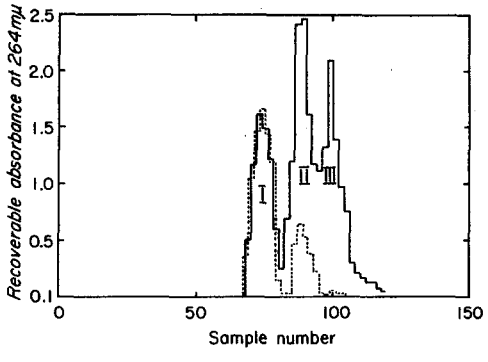

Fig. 2. Elution profile from Dowex 1 x 8 column, 2 x 40 cm. Solid line: Pattern obtained from a mixture of $\widehat{TT}$, $\widehat{TU}$ and $\widehat{UU}$ formed by UV irradiation of frozen thymine-uracil solution. Broken line: Pattern obtained from a dimer sample from UV irradiated DNA.

in solution, it could be shown that the first peak was $\widehat{TT}$, the second was $\widehat{TU}$ and the third was $\widehat{UU}$.

The dimer fraction from DNA, separated on this column gave two peaks, coinciding with the $\widehat{TT}$ and the $\widehat{TU}$.

A third very small peak seemed to occur in the region where the UU should have appeared, but the amount of material was too small for further unequivocal identification.

IR spectra were taken of:

1) a $\widehat{TT}$ sample from a UV irradiated frozen thymine solution;

2) a $\widehat{TT}$ sample from a UV irradiated frozen thymine-uracil solution, and

3) a $\widehat{TT}$ sample from a UV irradiated DNA solution.

These three spectra were identical (one is shown in Fig. 3a) showing that the TT from these three sources are the same compounds.

IR spectra were also taken of:

4) a $\widehat{TU}$ sample from UV irradiated frozen thymine-uracil solution, and

5) a $\widehat{TU}$ sample from UV irradiated DNA.

Both spectra were identical (one is shown in Fig. 3b) showing that the TU from these two sources are the same compound.

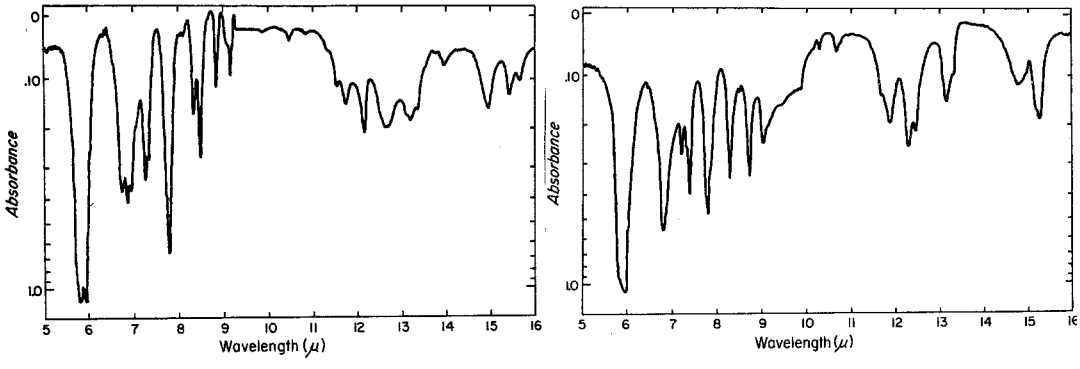

Fig. 3. (a) Infra Red Spectrum of $\widehat{TT}$, (b) of $\widehat{TU}$.

We now give indirect evidence to support the idea that the $\widehat{TU}$ dimer

resulting from the deamination of $\widehat{TC}$ produced in DNA also has the configuration of Fig. 1. In the case of thymine dimers, Weinblum and Johns, 1966, separated the four possible isomeric forms on the column described in Methods 1b. No attempt has been made to generate and separate the four possible dimers of either $\widehat{TU}$ or $\widehat{UU}$. While we do not have unequivocal proof that a mixture of such dimers would be separable under the same conditions described in Methods 1b or 1d, the resolving power of the columns is such that any isomers of $\widehat{TU}$ or $\widehat{UU}$ should have been separable. Furthermore, since the $\widehat{TU}$ from DNA is identical to the $\widehat{TU}$ from frozen solution, neither could be a mixture of two isomers unless one makes the very improbable assumption that the proportions of the mixtures be the same from two independent sources. Thus $\widehat{TU}$ represents only one isomeric species. We now argue that the $\widehat{TU}$ has the configuration of Fig. 1 since it elutes on a very long column (Method 1d) at almost the same place as $\widehat{TT}$.

Moreover, the formation of one particular $\widehat{TT}$ in high yield in frozen thymine solution is most convincingly explained by the assumption that the thymine molecules are lined up in a particular way in the ice lattice, allowing the formation of just one $\widehat{TT}$ isomer (Wang, 1961). When one half of the thymine in the ice lattice is replaced by uracil the overall yield of dimers is still high, and the ratio of $\widehat{TT}$: $\widehat{TU}$: $\widehat{UU}$ is 1:2:1. This result can best be explained by the assumption that uracil is aligned in the ice in exactly the same way as thymine. Consequently, $\widehat{TU}$ should have the same configuration as $\widehat{TT}$. This means that the thymine-cytosine dimer in DNA is also formed from neighbour molecules in one strand and is of the type shown in Fig. 1.

ACKNOWLEDGEMENTS

This study was supported by grants from the Medical Research Council of Canada and the National Cancer Institute of Canada. The author is indebted to Dr. H.E. Johns for continual support and interest in this project.

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