Photodimer of Thymine in Ultraviolet-Irradiated DNA: Proof of Structure by X-ray Diffraction

Abstract. *Dimethylthymine was photodimerized with ultraviolet radiation, and the structure of one of the resulting dimers, the* cis *5,5 : 6,6 isomer, was determined by single-crystal x-ray diffraction. This isomer is the same as that obtained by ultraviolet irradiation of DNA; hence the structure of the thymine dimer in irradiated DNA is now proved.*

Dimers of thymine were first isolated and identified as ultraviolet photoproducts from frozen aqueous solutions of thymine and from aqueous solutions of DNA (*1*). Wulff and Fraenkel (*2*) pointed out that *cis*-linkage of two thymines to a cyclobutane ring formed across their 5,6 double bonds would give rise to four stereoisomeric dimers (Fig. 1). Four different isomers have been obtained (*3*), and a number of assignments of their structures from chemical, spectroscopic, and crystallographic speculations have recently culminated in the detailed x-ray determinations of the structures of the 5,6:5,6 *trans* dimers of thymine (*4*) (isomer IV, Fig. 1) and 1-methylthymine (*5*).

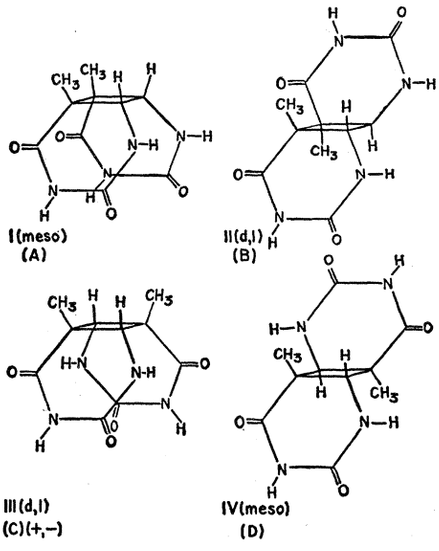

Fig. 1. The four possible stereoisomeric photodimers of thymine, according to Wulff and Fraenkel (*2*).

Only one isomer is obtained from the ultraviolet irradiation of DNA. Chemical and spectroscopic investigation has shown (*2*, *6*) that one of the two dimers obtained from irradiation of 1,3-dimethylthymine is the same isomer as the photodimer from DNA. We have solved the crystal and molecular structure of this dimer of dimethylthymine, and present unequivocal proof of the conformational structure of the thymine photodimer formed upon ultraviolet irradiation of DNA.

Photodimer A [in the notation of (*3*)] of dimethylthymine was prepared by ultraviolet irradiation of a frozen solution of dimethylthymine, and crystals were grown from aqueous solution. The crystals are monoclinic with a = 8.41, b = 14.01, c = 14.55 Å, β = 117°15′; space group $P2_1/c$, with Z = 4 dimers per cell. A full set of three-dimensional intensities was measured on a Picker automatic four-circle diffractometer with a scintillation counter and CuK_α radiation. The test intensities and cell constants of the crystal changed slowly with x-ray exposure; this suggested to us that the x-rays were monomerizing the dimer in the crystal, and this was confirmed by the presence of monomer peaks in the ultraviolet spectra of the dimer after prolonged x-ray irradiation. This effect is also shown by the lengths of the cyclobutane-ring bonds *between* the two thymine residues; these bonds are much longer than those normally found [greater than 1.65 Å at this stage of refinement, as against 1.58 Å in the 5,6:5,6 *trans* dimers (*4*, *5*)], indicating the partial splitting of the 5,5 and 6,6 bonds during exposure to x-rays, and slow drift apart of the newly formed monomers.

The crystal structure was solved directly by the symbolic addition procedure (*7*), and all of the atoms were located on the first E-map, and their positions and thermal parameters were refined by the least-squares method. The current discrepancy factor is R = .113, and further refinement is needed before accurate bond distances can be obtained. Superimposed sections of the electron density calculated at the atomic centers are shown in Fig. 2, along with a perspective drawing of the molecule. The electron-density map confirms the 5,5:6,6 *cis* conformation of the dimer, as expected from the dimerization of adjacent thymines in DNA.

The angle between the best planes through all six atoms of the thymine residue nuclei is about 39°. The residues are not planar, but least-squares planes calculated through all combinations of five atoms taken at a time showed that one of the rings is planar if C-6 is omitted from the calculation (maximum deviation from plane = 0.02 Å, and deviation of C-6 from this plane = −0.33 Å), and the other thymine nucleus is planar if C-5 is omitted (maximum deviation = 0.03 Å; C-5 deviation = +0.44 Å). The angle between these planes is 28.5°.

The two thymine residues are rotated with respect to each other, the angle of rotation being about 28° (Fig. 2). Since this angular displacement is thought to be 36° for two adjacent thymines in DNA, only a small angular twist (about 4° for each thymine) probably accompanies photodimerization in DNA. The cyclobutane ring is markedly puckered: each carbon atom of the ring lies 0.6 Å out of the plane of the other three atoms.

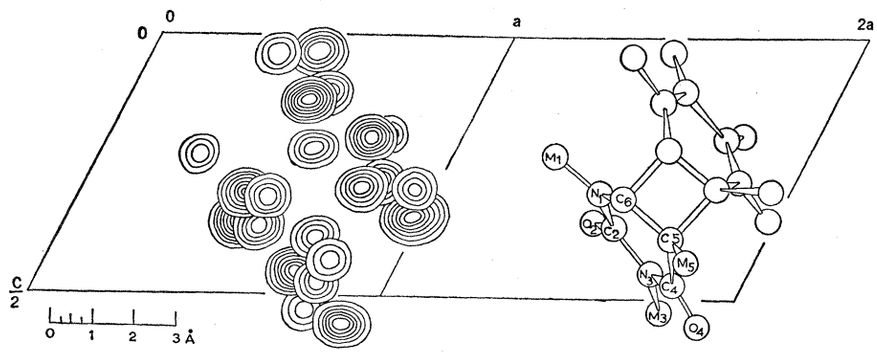

Fig. 2. Superimposed sections of the electron density calculated at the atomic centers, parallel to (010). C, Carbon; N, nitrogen; O, oxygen; M, methyl-group carbon.

NORMAN CAMERMAN
Department of Chemistry,
University of Toronto,
Toronto 5, Canada

ARTHUR CAMERMAN
Department of Biological Structure,
University of Washington,
Seattle, Washington 98105

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8. Most of the experimental work was performed at the University of Toronto, and we thank Professor S. C. Nyburg for the use of facilities in his laboratory and for encouragement. We also thank D. Weinblum for supplying the material.

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Pyrophosphate in the 5′ Terminal Position of a Viral Ribonucleic Acid

Abstract. *A pancreatic ribonuclease digest of carbon-14–labeled Satellite Tobacco Necrosis Virus RNA was fractionated, according to charge, by column chromatography. Individual fractions were dephosphorylated with alkaline phosphomonoesterase and rechromatogramed. The fraction originally containing oligonucleotides with seven negative charges separated into two components corresponding to five and two negative charges, respectively, and therefore must have contained a terminal trinucleotide 5′-pyrophosphate, in addition to the internal hexanucleotides. Other fractions when similarly treated were found to contain only internal oligonucleotides.*

The 5′ hydroxyl end (*1*) of naturally occurring RNA (*2*) molecules is most commonly found to be esterified to a monophosphate (*3*). Yet it has recently been shown that bacteriophage R17 RNA contains a triphosphate in the 5′ position (*4*). This latter finding is consistent with current ideas on the mechanism of RNA synthesis. The presence of a monophosphate in the 5′ position in many RNA's may therefore be a result of a subsequent cleavage of a triphosphate, suggesting that in vivo the 5′ end of RNA's may be subject to specific and limited dephosphorylations. Thus, limited and specific dephosphorylation of certain species of RNA could lead to the removal of a single phosphate group, resulting in an RNA containing a pyrophosphate at the 5′ end.

This report presents evidence that Satellite Tobacco Necrosis Virus RNA (STNV RNA), as prepared in our laboratory, contains such a 5′ terminal pyrophosphate group.

Virus preparations were made from Turkish Tobacco plants (variety Xanthi), which were grown in a chamber in an atmosphere containing 10 mc of $^{14}CO_2$. The virus was isolated as described (*5*), by using nonradioactive infected plant extracts as carrier in sufficient quantities to avoid the loss of labeled virus. The purified virus solution was adjusted to 0.1*M* $(NH_4)_2CO_3$ immediately prior to isolation and purification of the RNA (*6*). Ribonucleic acid from STNV (180 O.D. units, 790,000 count/min) was digested at 37°C for 24 hours in 0.1*M* tris HCl buffer, *p*H 7.5, with 0.6 mg of pancreatic ribonuclease (RAF, 7da, phosphate free, Worthington, Freehold, N.J.). For column chromatography, DEAE-cellulose (Cellex D, Bio-Rad, Richmond, Calif.) was purified by repeated washing with 3*M* NaCl and then 2*M* $(NH_4)_2CO_3$. Columns for the separation of enzyme digests were packed under slight pressure (⅓ atm) with this DEAE-cellulose, equilibrated in 7*M* urea and 0.01*M* tris HCl buffer, *p*H 7.5. The columns were then eluted with a linear gradient of NaCl in 7*M* urea, 0.01*M* tris HCl buffer, *p*H 7.5. Additional details are given in the figure legends. Oligonucleotides eluted from this column were freed of salts and urea by dilution with 4 volumes of H_2O, passage onto small (1- by 10-cm) DEAE-cellulose (carbonate form) columns equilibrated in 0.005*M* triethylammonium carbonate (TAC) (*7*) and subsequently eluted with 1*M* TAC. These fractions were then flash evaporated and lyophilized overnight to remove TAC. Each lyophilized fraction was dissolved in 1 ml of 0.1*M* tris HCl buffer, *p*H 8, and incubated for 2 hours at 37°C with 0.3 unit of *E. coli* alkaline phosphatase (BAPF, ribonuclease free, Worthington, Freehold, N.J.) per O.D. unit of oligonucleotide. Crystalline urea was then added to each mixture to a final concentration of 7*M* and, following the addition of appropriate markers, the sample was reapplied to DEAE-cellulose (chloride form) columns, and eluted with a NaCl gradient as above.

Aliquots of aqueous solutions were mixed with 15 ml of Bray's scintillation liquid (*8*) and counted for radioactivity in a Beckman LS-250 scintillation counter.

Figure 1 shows an elution profile of a pancreatic ribonuclease digest of STNV RNA. The oligonucleotides are separated on DEAE-cellulose according to their charge in 7*M* urea (*9*). Fractions −5, −6, −7, and −8 (numbers representing the number of negative charges) were desalted, dephosphoryl-

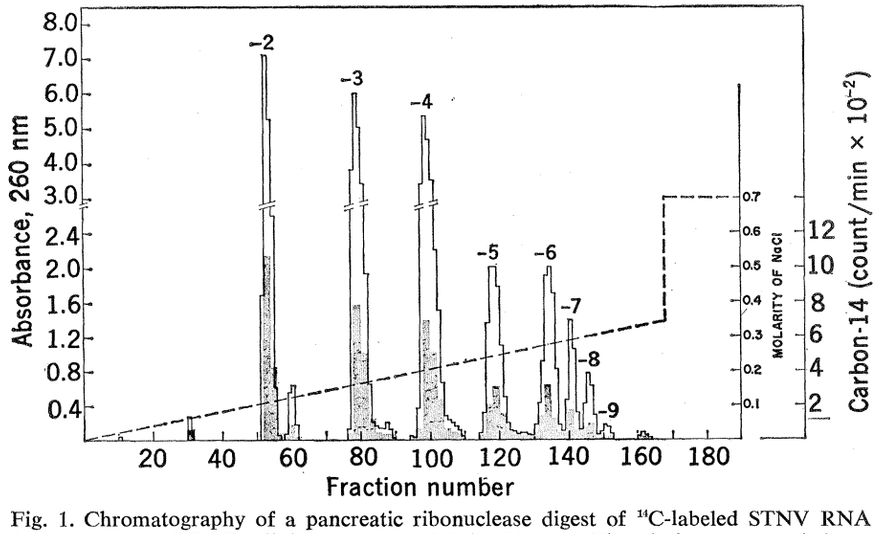

Fig. 1. Chromatography of a pancreatic ribonuclease digest of ^{14}C-labeled STNV RNA (see text) on a DEAE-cellulose column (0.8 × 55 cm). The elution was carried out with a gradient of NaCl concentration (broken line) in 7*M* urea, 0.01*M* tris HCl; total volume 500 ml. Fractions of 3 ml were collected. Aliquots of each fraction were measured for absorbance at 260 nm (solid line) and radioactivity (shaded area). The numbers above the peaks represent the number of charges of each oligonucleotide eluted in the peak.