Photochemical Decomposition of TpBU

By A. Haug

Aus dem Institut für Genetik der Universität Köln
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Irradiation of the dinucleotide TpBU at all wavelengths from 240 to 295 mµ produces a debranomated photoprocess and two minor products. The main compound has an absorption maximum exactly at the same wavelength as the parent compound, but with approximately half the value of the extinction coefficient. It cannot be reverted by short wavelengths, such as 240 mµ.

If 5-bromouracil instead of thymine is incorporated into the DNA, the irradiated organisms (phages, bacteria, animal cells) apparently are more susceptible towards the lethal effects of ultraviolet light. The enhanced inactivation depends upon the fact that with increasing BU-substitution of the DNA the host-cell reactivation decreases¹. It is also of biological interest that UV damages in BU-DNA cannot be reverted by the photoreactivating enzyme².

Quantitative statements about the courses of the photochemical reactions involved are not discussed in the literature. Qualitatively several authors have detected uracil after BU-DNA was irradiated with ultraviolet light and subsequently acidhydrolyzed³.

Bromouracil UV-irradiated at —20 °C in the presence of cytosine, uracil, and uridine yields several debrominated and bromine containing photoproducts. Bromouracil irradiated in ice does not show any photochemical alteration, in contrast to thymine⁴.

The behavior of dinucleotides approximate that of the native DNA more closely than the free base or the mononucleotide. Therefore it seemed reasonable to us to study the effect of UV radiation on the dinucleotide TpBU and to compare its photochemistry with that of the TpT, for which somewhat more detailed investigations are available⁵ ⁶ ⁷. If TpT is irradiated with ultraviolet light in aqueous solution, a steady state can be obtained by choice of the appropriate wavelength. At the “long” wavelength, 285 mµ, the steady state lies at the intramolecular dimer TpT characterized by a cyclobutane structure. The “short” wavelength, 240 mµ, splits this ring structure and the steady state lies at over 90% TpT. Presently available results indicate that UV-irradiated TpT yields only its intramolecular dimer. TpBU differs from TpT in the substitution of a bromine atom for the methyl group; the bromine having approximately the same van der Waals’ radius as the methyl group. Furthermore, since TpBU contains thymine it should be a favourable substance for study because thymine, unlike cytosine and uracil, does not undergo photohydration. The simultaneous presence of this photohydration would considerable complicate the understanding of the other photochemical reactions.

Material and Methods

The irradiations were carried out with a water prism UV-monochromator. This instrument is a copy of the monochromator first constructed at the California Institute of Technology, Pasadena, and improved in our Institute. The light source was an air-cooled high pressure mercury lamp (Philips Sp 900 W) the power supply of which was magnetically stabilized. The power of the light beam impinging on the sample amounts to about 1.6 × 10³ erg/sec at 270 mµ, with a slit width of about 10 mµ. The intensity of the beam was actinometrically calibrated vs. wavelength by malachite green leucocyanide.

The samples were irradiated at room temperature in a normal Beckman cell (1 cm depth, 3 ml vol.) with mechanical stirring. We did not deaerate the solutions. To measure the quantum yield we used TpBU concen-

¹ W. Sauert & Der, Virology 17, 164 [1962].
⁷ R. A. Deering and R. B. Setlow, personal communication.

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trations of about $10^{-4} \text{m}$ in $m/100$-phosphate buffer ($pH$ 7). For preparative work we irradiated batches of about 100 OD units (= 12 mg, OD = optical density) and followed the course of the reaction, after adequate dilution, with the spectrophotometer.

All absorption measurements were carried out with a Cary-15 spectrophotometer. $pH$-measurements were performed with a $pH$-meter 22 of Radiometer, Inc., Copenhagen. High-voltage electrophoresis was carried out for $3^{1/2}$ hours at 2 KV, 120 mA in 0.05-m ammonium acetate buffer with Whatman 3 mm chromatography paper. Descending paper chromatography was done on Whatman 3 mm paper in isopropanol/$\text{NH}_3$/$\text{H}_2\text{O} = 7 : 1 : 2$ (V/V).

**Synthesis of TpBU**

TpBU was prepared in analogy to TpT according to the method described by Gilham and Khorana except that thymidine was replaced by bromodeoxyuridine. The starting materials, thymidylid acid and BUDR, were purchased from California Corp. for Biochemical Research, Los Angeles, or from Schwarz BioResearch, Orangeburg.

The synthesized product was first paper-chromatographed and a band with the same $R_f$-value as TpT (0.45) was subjected to an additional high voltage electrophoresis. Here two bands were visible. The slower one was TpBU; the faster band was thought to be a pyrophosphate and, therefore, was not examined further. TpBU was eluted from the paper with water and lyophilized. To measure the extinction coefficient a sample was dried in a vacuum tank over phosphorus pentoxide and weighed. A paper blank due to elution of the paper sheet was subtracted.

**Results**

**Spectral properties of synthesized TpBU.** The ultraviolet absorption spectrum of TpBU is plotted at $pH$ 2, 7 and 12 in fig. 1 and characteristic spectral data of TpT at $pH$ 7 and TpBU at $pH$ 7 and 12 are listed in Tab. 1. The absorption spectrum varies only slightly between $pH$ 2 and 8. At more alkaline $pH$ values the molar extinction coefficient $\epsilon_{max}$ decreases and $\lambda_{max}$ increases and shifts to longer wavelengths. Measurements at several $pH$'s between $pH$ 2 and $pH$ 12 show that TpBU has an isosbestic point at 255 m$\mu$. This spectral behaviour can be attributed to ionizations of the 6-carbonyl groups of the participating bromouracil and thymine moieties.

**Photoproducts of TpBU.** Buffered TpBU solutions with an optical density of approximately 100 OD units at 271 m$\mu$ were maximally irradiated at 275 m$\mu$. Afterwards the solution was lyophilized and the residue was separated by high voltage electrophoresis. Under the fluorescence lamp three bands were visible. These three bands have been labelled I, II, and III in the direction of their migration from $-$ to $+$. Each band was cut out, eluted with water, and lyophilized. Band III accounted for 90% by weight of the starting material, band I and II accounted for 4% each.

**Photoproducts I and II.** Photoproduct I shows no detectable migration in the electric field and is clearly separated from photoproduct II. The latter runs about 1/3 as fast as the main band III. In Tab. 2 the spectral data of I and II are listed.

**Photoproduct III.** Upon 240 m$\mu$ radiation, the OD$_{max}$ (271 m$\mu$) of the solution eluted from the

<table>
<thead>
<tr>
<th>$pH$</th>
<th>$\lambda_{max}$ [m$\mu$]</th>
<th>$\lambda_{min}$ [m$\mu$]</th>
<th>$\epsilon_{max}$</th>
<th>$\epsilon_{min}$</th>
<th>$\epsilon_{max}$</th>
<th>$\epsilon_{min}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TpBU</td>
<td>7.0</td>
<td>271.4</td>
<td>239.5</td>
<td>17800</td>
<td>5500</td>
<td>3.25</td>
</tr>
<tr>
<td>TpT</td>
<td>12.0</td>
<td>270.0</td>
<td>249.0</td>
<td>14200</td>
<td>9100</td>
<td>1.55</td>
</tr>
</tbody>
</table>

Tab. 1. Spectral properties of TpBU and TpT.

![Fig. 1. Spectrum of TpBU at different pH values.](image-url)
electrophoresis band shows practically no decrease within reasonable doses of irradiation. Irradiation of photoproduct III with 275 m\(\mu\) light causes a gradual decrease in OD\(_{\text{max}}\) with a quantum yield of \(\varphi \ll 0.001\).

The \(p_H\) dependence of the spectrum of compound III is plotted in fig. 2 and Tab. 2 lists the characteristic spectral data of this photoproduct. Compound III and TpBU have very similar absorption spectra. At more alkaline \(p_H\) s the ratio \(\epsilon_{\text{max}}/\epsilon_{\text{min}}\) is much smaller than with TpBU. \(\lambda_{\text{max}}\) shows a hypsochromic shift by 4 m\(\mu\) and \(\lambda_{\text{min}}\) shows a bathochromic shift of 16 m\(\mu\).

Microanalysis on halogens in this compound shows 2.5% organically bound halogen. Since the dinucleotide TpBU has 12% halogen, the 2.5% halogen present can very probably be explained as paper contamination from electrophoresis and chromatography. We conclude that compound III contains no bromine.

<table>
<thead>
<tr>
<th>photoproduct</th>
<th>(p_H)</th>
<th>(\lambda_{\text{max}}) [m(\mu)]</th>
<th>(\lambda_{\text{min}}) [m(\mu)]</th>
<th>(\epsilon_{\text{max}}/\epsilon_{\text{min}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7</td>
<td>266</td>
<td>242</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>7</td>
<td>276</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>271.5</td>
<td>239.0</td>
<td>3.63</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>271.5</td>
<td>239.0</td>
<td>3.53</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>267.5</td>
<td>255.5</td>
<td>1.13</td>
</tr>
</tbody>
</table>

Tab. 2. Spectral properties of TpBU photoproducts.

Dependence of the irradiated solution of TpBU on \(p_H\), temperature, and silver nitrate. If one adds 0.01 ml of 12-m. NaOH or HCl to 3 ml of a 10\(^{-4}\)-m. solution of TpBU which was irradiated up to a maximum change at 271 m\(\mu\), an instantaneous and reversible spectral change was observed, similar to the variation of the spectrum of compound III at different \(p_H\) values.

Addition of 0.1 ml of a 5% solution of AgNO\(_3\) to a 10\(^{-3}\)-m. solution of TpBU irradiated to a maximum change at 271 m\(\mu\), results in a fluffy turbidity characteristic for AgBr.

If a solution is irradiated to maximum change at 271 m\(\mu\) and then heated for 10 min. at 85°C, there is no detectable alteration in the spectrum. The spectrum also remains constant upon standing for 24 hours at room temperature.

**Dependence of the quantum yield on wavelength.**

At all wavelengths used, the optical density at 271 m\(\mu\) of a buffered solution of TpBU decreases to half its value upon exhaustive irradiation and then decreases slowly. There is no wavelength shift of OD\(_{\text{max}}\). A semilogarithmic plot in fig. 3 shows the rate of decrease of OD\(_{\text{max}}\) with the dose of 265 m\(\mu\) radiation. The observed curve shows a fast decrease and a slow decrease. The fast photochemical reaction corresponds with the decomposition TpBU \(\rightarrow\) III, the slow one to the process III \(\rightarrow\) ? (see p. 146).

The dependence of the initial quantum yield of the photochemical decomposition of TpBU (as calculated below) as a function of wavelength is graphed in fig. 4. This quantum yield represents the sum of partial quantum yields according to the different photochemical reactions. We could not detect any dependence of the quantum yield on the concentra-
tion of the TpBU in buffered solution ($5 \times 10^{-5}$-m. to $5 \times 10^{-3}$-m.) The $p_H$ dependence was not tested. Such a dependence might be possible because halogenated uracils and their corresponding nucleosides exist to an appreciable extent in both ionized and un-ionized forms in neutral aqueous solution. The pH dependence was not tested.

Fig. 4. Dependence of the quantum yield of TpBU on wavelength.

A solution of TpBU, irradiated at 285 m$\mu$ until OD$_{\text{max}}$ had dropped to near its minimum value, was subsequently irradiated at 240 m$\mu$. About 10% of the original drop in OD$_{\text{max}}$ was recovered. Further irradiation at 285 m$\mu$ gave a drop in OD$_{\text{max}}$, again to its minimum value, and subsequent irradiation at 240 m$\mu$ again gave a recovery of about 10 per cent. This procedure could be repeated several times with the same results. From this we conclude that the two reactions considered are independent of each other, and that the 240 m$\mu$ reversal is probably due to small amounts of contaminating substance whose paper chromatographic behavior is very similar to that of TpBU.

Reaction kinetics. We saw that irradiation of the dinucleotide TpBU with ultraviolet light predominantly produces photoproduct III, which has at 271 m$\mu$ half the molar extinction coefficient of the original compound. The remaining photoproducts, which occur in smaller quantities at only higher doses, will not be considered here. On the basis of our observations we, therefore, have the following mode of decomposition:

$$\text{TpBU} \xrightarrow{\varepsilon} \text{III} \xrightarrow{\varepsilon/2} \text{large doses} \xrightarrow{?}$$

Let $C_T$ be the molar concentration of TpBU, $C_{\text{III}}$ that of compound III, $C_0$ the constant total concentration. Then for all doses of irradiation we have, when $L$ the total incident average flux:

$$C_0 = C_T(L) + C_{\text{III}}(L). \quad (1)$$

Let us call $D_{\text{tot}}$ the optical density at 271 m$\mu$ (the wavelength at which the reaction was followed) of the mixture of TpBU and compound III, measured at different doses of irradiation. Then

$$D_{\text{tot}} = \varepsilon_T \cdot C_T + \varepsilon_{\text{III}} \cdot C_{\text{III}}. \quad (2)$$

From eq. (1) and (2), when $\varepsilon_{\text{III}} = \varepsilon_T/2$, it follows for the concentration of TpBU

$$C_T(L) = \frac{2 \varepsilon_T}{\varepsilon_T} \cdot D_{\text{tot}} - C_0. \quad (3)$$

If $A$ is the reaction rate constant of the decomposition of TpBU, then we get at the start of irradiation:

$$\left( \frac{dC_T}{dL} \right)_{L=0} = -A \cdot C_T(0). \quad (4)$$

The constant $A$ may be calculated by use of eq. (3) and (4)

$$\left( \frac{dD_{\text{tot}}}{dL} \right)_{L=0} = -A \cdot \frac{\varepsilon_T}{2} \cdot C_T(0). \quad (5)$$

The quantum yield $\varphi$ may be determined by the following relationship:

$$A = 2.303 \cdot 10^3 \cdot \varepsilon_T \cdot \varphi. \quad (6)$$

Here $\varepsilon_T$ equals the molar extinction coefficient of TpBU at the wavelength irradiated.

Discussion

Our experiments demonstrate that the photochemistry of TpBU is different from that of TpT. Exhaustively irradiated TpBU yields one main photoproduct and several by-products. TpT, however, forms only its intramolecular dimer according to the present state of knowledge. The essential differences of the photoproduct of TpT compared to the main compound III of TpBU are listed as follows:

1. Compound III has an absorption maximum at the same wavelength as the parent compound, but with half the value. TpT has $\varepsilon \approx 0$ at 271 m$\mu$ and $p_H 7$.

2. Compound III cannot be reverted to the parent compound by irradiation with 240 m$\mu$ whereas TpT is reverted with the quantum yield $\varphi = 0.43$. According to their electrophoretic migration photoproduct I might be a nucleoside, compound II
a nucleotide. $\lambda_{\text{max}}$ of compound II is at 276 m$m\mu$, implying that it is a molecule containing bromine. For example, 5-BUDR at $p_H$ 7 shows $\lambda_{\text{max}}$ at 278 m$m\mu$, while the corresponding thymine derivative has $\lambda_{\text{max}}$ at 267 m$m\mu$.

Further investigations are necessary to decide whether compounds I and II arise from TpBU or from III. In this connection Greer and Zamenhof ¹⁰ demonstrated that the sugar portion of 5-BUDR is split away by heating to 100 °C ($p_H$ 7). The corresponding riboside, 5-BUR, shows no detectable splitting under the same conditions. It is conceivable that one of the further decomposition products arises from breaking the sugar bond.

About 90% of TpBU are converted into the main photoprodut III independent on the wavelength used. Even raising the concentration of the irradiated solution of TpBU by a factor of 100 did not alter the relative amounts of III and photoproducts I and II. The independence of the quantum yield on the concentration and the migration of TpBU upon paper chromatography point to a dimer. TpBU and TpT have an $R_f$ value of about 0.45 each, III and TpT values of 0.33 and 0.31 respectively. Because the extinction coefficient amounts to 9000 in the debrominated molecule, there must exist one set of conjugated double bonds. The only reasonable structure consistent with these considerations is an intramolecular dimer with cyclobutene structure. Let us designate it with TpBU.

![Fig. 5. The conjectured structure of the principal photoprodut TpBU.](image)

Of some biological interest is that compound III cannot be reverted to its parent compound, in contrast to TpT.

The author is deeply indebted to Dr. M. Delbrück for many stimulating discussions and criticism and to Dr. S. Y. Wang for much advice with the synthesis of the dinucleotides. Thanks also to Drs. P. Parker and D. L. Wulff for helpful discussions.

¹⁰ S. Greer and S. Zamenhof, J. molecular Biol. 4, 123 [1962].