Photosensitized formation of thymine dimers in DNA by tyramine, tyrosine and tyrosine-containing peptides

Motohisa Kaneko, Akio Matsuyama and Chikayoshi Nagata

Biophysics Division, National Cancer Center Research Institute, Tsukiji 5-1-1, Chuo-ku, Tokyo 104, Japan

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ABSTRACT
The formation of Thy-Thy in DNA in the presence of tyramine, tyrosine and tyrosine-containing peptides such as Lys-Tyr and Lys-Tyr-Lys was studied with monochromatic UV irradiation. The formation of Thy-Thy by UV irradiation was enhanced in the presence of these compounds. The action spectrum of the photosensitization has a peak near 280 nm corresponding to the absorption spectrum of tyrosine. The triplet quencher reduced the sensitization substantially. The sensitization in native DNA was more than six times larger than that in denatured DNA. Increasing the concentration of salts suppressed the sensitization. The nature of the interaction between DNA and the sensitizer is discussed.

INTRODUCTION
There are several lines of evidences that protein is a target for the inactivation of cells (1). Smith proposed the hypothesis that DNA-protein cross-linking contributed to the inactivation and showed the parallelism between the killing of cells and the loss of extractability of DNA (1). Concerning the participation of protein in the inactivation of cells, we proposed the possibility that the formation of Thy-Thy in DNA by UV irradiation was sensitized by tyrosine residues by the triplet-triplet energy transfer from tyrosine to Thy (2). This hypothesis is based on the triplet-triplet energy transfer from histone to DNA in deoxyribonucleohistone observed by the phosphorescence spectra (3) and it was verified in the case of Thy monomer and tyrosine in aqueous solution (2). In the present work, we observed the photosensitized formation of Thy-Thy in DNA in vitro by tyramine, tyrosine and tyrosine-containing peptides.
MATERIALS AND METHODS

CHEMICALS, Radioactive \(^{14}\text{C}\)-thymine (61 mCi/ml) was purchased from the Radiochemical Center, Amersham, U.K. Unlabelled thymine, p-methoxyphenylethylamine were purchased from Sigma Chem. Co.; tyramine, p-ethylphenol from Tokyo Kasei Co; tyrosine from Ajinomoto Ltd, Tokyo; Lys-Tyr, Lys-Tyr-Lys from Bachem Inc, California.

DNA, Labelled DNA (\(^{14}\text{C}-\text{Thy}\)) was prepared from E.coli W3110 (thy\(^{-}\)) by the SDS-phenol method. The \(A_{280}/A_{260}\) of DNA was 0.543. The labelled DNA has the specific activity of 6.3 nCi/nmol in nucleotide and was stored at 4°C in 10 mM Tris-HCl (pH 7.4).

UV IRRADIATION, The monochromatic ultraviolet radiation was obtained by use of a grating monochromator MC-10N of Rittsu Applied Optics, Tokyo, illuminated by a 1 KW Xe short arc lamp of Ushio Electric Co., Tokyo, 0.5 ml samples of DNA (44.5 \(\mu\)M in nucleotide) in 10 mM Tris-HCl (pH 7.4) were irradiated in a quartz micro-cell (1.0 cm path length) placed at the exit slit of the monochromator. Intensities were measured by a chemical actinometer of potassium ferrioxalate (4). At 280 nm (+ 4 nm) the fluence rate at the exit slit was 13 W/m\(^2\). Stirring and deoxygenation were carried out by bubbling nitrogen through the solution. Isoprene carried by nitrogen was bubbled instead of nitrogen if necessary.

ANALYSIS OF PHOTOPRODUCTS, Irradiated DNA samples were dialysed against distilled water, evaporated to dryness and dissolved in formic acid. Acid hydrolysates were chromatographed on Whatman No. 1 paper by descending technique. The solvent system used was n-butanol/acetic acid/water (80:12:30, v/v/v). The strips were cut in 1 cm pieces and \(^{14}\text{C}\) label was counted by Packard liquid scintillation counter. Characterization of products is according to Patrick and Rahn (5).

RESULTS

PHOTOPRODUCTS AND FLUENCE DEPENDENCE, Figure 1 shows the fraction of Thy present as Thy\(^{\bullet}\)Thy in UV irradiated E.coli DNA at different concentration of tyramine and at different fluence (in terms of the average fluence in the sample) at 280 nm. The
The fraction of Thy present as ThyOThy at different concentration of tyramine at 280 nm.

quantum yield for ThyOThy formation was 0.015 according to the approach of Deering and Setlow (6). This is almost in agreement with those published by others (5). In the presence of tyramine, the amount of ThyOThy produced increases as increasing the concentration of tyramine. This shows that the formation of ThyOThy in DNA is photosensitized by tyramine.

The fraction of Thy present as ThyOura (from deamination of ThyOCyt formed in situ) was about one sixth of that of ThyOThy in the absence of tyramine (data not shown). In the presence of 0.52 mM tyramine, the cross section for ThyOura formation increased only 45% (data not shown). Since the increase in ThyOThy formation was about 280% in the same samples, ThyOura formation was less photosensitized by tyramine than ThyOThy formation. The fraction of Thy(6-4)Pyo which is indistinguishable chromatographically from that of ThyOThy in the present solvent system was neglected, because Thy(6-4)Pyo is unstable in formic acid hydrolysis (7). Thus, we focused our investigation on ThyOThy formation.

ACTION SPECTRA. In order to confirm the contribution of the
electronically excited state of tyramine to the sensitized formation of Thy\*Thy in DNA, we obtained the action spectrum for the formation of Thy\*Thy. Figure 2 shows the action spectra in the absence or presence of 0.52 mM tyramine. The action spectrum in the absence of tyramine is corresponding to the absorption spectrum of Thy in agreement with that of Murphy (8). The action spectrum for the sensitized formation of Thy\*Thy is corresponding approximately to the absorption spectrum of tyramine. These results are in agreement with those for the sensitized formation of Thy dimers in aqueous Thy solution (2). Therefore, the electronically excited states of tyramine contribute to the sensitized formation of Thy\*Thy in DNA.

**EFFECTS OF ISOPRENE,** On the basis of the above results, we can conclude that the energy transfer from tyramine to DNA bases is responsible for the photosensitization. In order to clarify the involvement of the triplet state of tyramine or Thy in the sensitization, we examined the effects of a triplet quencher on the sensitized formation of Thy\*Thy. As a triplet quencher, we used isoprene which is bubbled with nitrogen in the sample (the concentration of isoprene in the sample was 0.7 mM). Figure 3

![Figure 2. Action spectra in the absence (O) or presence (●) of 0.52 mM tyramine.](image-url)
Figure 3. Effects of isoprene on the sensitization. Circle: nitrogen bubbled, triangle: isoprene carried by nitrogen bubbled. Irradiated at 280 nm. The concentration of isoprene in the sample is 0.7 mM.

shows the cross section for the formation of Thy^Thy in DNA at several concentrations of tyramine in the absence or presence of isoprene. In the presence of isoprene, the sensitization is markedly reduced at every concentration of tyramine. In the case of direct photoirradiation to DNA, however, the cross sections are not changed by the presence or absence of isoprene. The result is in accord with the observation that the yield of Thy^Thy is independent on paramagnetic metal ions (9). These observations suggest, firstly, that Thy^Thy are formed via the excited singlet state of Thy in DNA in the case of direct photoirradiation. Secondly, the triplet state of Thy contributes to Thy^Thy formation in the case of tyramine-sensitization.

EFFECTS OF SALTS. Since triplet-triplet energy transfer demands close proximity of energy donor and acceptor molecules, these molecules must interact in the range of Van der Waals radii. To clarify the nature of the interaction between energy donor and acceptor molecules in the sensitization, we investigated the effects of salts, the effects of the conformation of DNA and further, the effects of the structure of the sensitizer, i.e. the substitution of ethylamino group of tyramine by ethyl group and
the substitution of OH group of tyrosine ring by methoxy group.

Figure 4 shows the effects of NaCl or (NH₄)₂SO₄ on the cross section for the formation of Thy-Thy on changing their concentrations. In the absence of tyramine, the cross sections are almost invariable by changing the concentration of NaCl in agreement with Patrick (10). In the presence of tyramine, on the contrary, Thy-Thy formation is profoundly influenced by the changing concentration of salts, i.e. the cross section decreases steeply at lower concentration of NaCl (< 0.2 M) but decreases slowly at higher concentration of NaCl (> 0.2 M). Although the sensitization at 2.0 M NaCl remains about 40% of that in the absence of the salt, it almost disappears at 0.5 M (NH₄)₂SO₄. Thus the sensitization is dependent on the ionic strength of the solution.

DEPENDENCE ON DNA CONFORMATION. The interaction between DNA and small molecules is strongly dependent on the conformation of DNA (11). If the sensitization accompanies a specific interaction between DNA and tyramine, we can expect that the sensitization is also dependent on the conformation of DNA. Table 1

![Figure 4. Effects of salts. Circle: effects of NaCl in the absence (○) or presence (●) of 0.52 mM tyramine. Triangle: effects of (NH₄)₂SO₄ in the absence (△) or presence (▲) of 0.52 mM tyramine. Irradiated at 280 nm.](image-url)
<table>
<thead>
<tr>
<th>Sensitizer</th>
<th>native DNA (44.5 μM in nucleotide)</th>
<th>denatured DNA (44.5 μM in nucleotide)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\sigma(1) \times 10^{19}$</td>
<td>$\sigma / \sigma_o - 1$</td>
</tr>
<tr>
<td>Control</td>
<td>1.61</td>
<td>1.73</td>
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<tr>
<td>Tyramine</td>
<td>5.29</td>
<td>2.29</td>
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<td>p-Ethylphenol</td>
<td>2.73</td>
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<td>p-Methoxyphenyl-ethylamine</td>
<td>7.94</td>
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<tr>
<td>Tyrosine</td>
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<td>0.47</td>
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<tr>
<td>Lys-Tyr</td>
<td>2.74</td>
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<tr>
<td>Lys-Tyr-Lys</td>
<td>2.17</td>
<td>0.35</td>
</tr>
</tbody>
</table>

(1) The unit of $\sigma$ is in (cm$^2$/quantum). $\sigma_o$ is $\sigma$ of the control.

Irradiation; at 280 nm. 

shows the cross section for the reaction in native DNA and denatured DNA in the absence or presence of 0.52 mM tyramine. From the ratio of the cross section in the presence of a sensitizer ($\sigma$) to that in the absence ($\sigma_o$), we can obtain the relative efficiency of the sensitization at a given concentration of DNA and a sensitizer. Thus, we define the relative efficiency as $\sigma / \sigma_o - 1$. If there are no sensitization, the efficiency equals zero. The efficiency of the sensitization in denatured DNA is only about one sixth of that in native DNA. The hyperchromicity due to the denaturation of DNA was 41 % and this indicates that double-stranded portion in the denatured DNA is about 10 % on an average. Therefore, the photosensitization in single-stranded DNA is about one order smaller than that in double-stranded DNA.

**DEPENDENCE ON THE STRUCTURE OF THE SENSITIZER.** By the substitution of ethylamino group of tyramine by ethyl group, we can know whether the positive charge at the ethylamino group is essential for the sensitization. The formation of hydrogen bonding with nucleic acid bases was observed in OH group of p-cresol (the side chain of tyrosine) but not in methoxy group of p-cresol-methyl ester (12). Then, by the substitution of OH group of tyramine by methoxy group, we can get the information whether hydrogen bonding between DNA bases and the sensitizer is prerequisite for the sensitization.
Table 1 shows the cross sections for the reaction in the presence of 0.52 mM p-ethylphenol and p-methoxyphenylethylamine. These molecules also sensitize the formation of Thy•Thy. However, in the case of p-ethylphenol, the efficiency of the sensitization decreased to about one fourth of that of tyramine. Therefore, an electrostatic interaction contributes greatly to the sensitization, although not prerequisite. On the other hand, in the case of p-methoxyphenylethylamine, the efficiency is a little higher than that of tyramine. Therefore, hydrogen bonding by OH group of tyrosine ring seems not contributing to the sensitization.

Comparison between tyrosine and tyrosine-containing peptides as sensitizers. We examined the possibility of the sensitization by tyrosine residues in peptides using simple oligo-peptides. In addition to tyramine, tyrosine and tyrosine-containing peptides such as Lys-Tyr and Lys-Tyr-Lys also sensitize the formation of Thy•Thy in DNA at 280 nm. The cross sections for the reaction are shown in Table 1. The efficiency of the sensitization increases in the order of Lys-Tyr-Lys < tyrosine < Lys-Tyr < tyramine.

Discussions

We have observed the sensitized formation of Thy•Thy in DNA by tyramine, tyrosine and tyrosine-containing peptides. The sensitization was dependent on the concentration of tyramine (Fig. 1) and the action spectrum for the sensitization was similar to the absorption spectrum of tyramine (Fig. 2). Therefore, electronically excited states of tyramine contribute to the sensitization. Furthermore, the sensitization was suppressed by a triplet quencher, isoprene (Fig. 3). Consequently we concluded that the sensitization was due to the triplet-triplet energy transfer from tyramine to Thy. The formation of Thy•Ura (from deamination of Thy•Cyt formed in situ) was not sensitized by tyramine as much as that of Thy•Thy. In the case of the sensitization by acetone, which is due to triplet-triplet energy transfer, Cyt-containing products were also relatively fewer than those in direct photoirradiation (9). These results might be explained by the difference in the efficiency of energy transfer to Cyt and Thy. As for the formation of Thy(6-4)Pyo,
which we did not observe, there are strong reasons that the formation of Thy(6-4)Pyo would not be sensitized by tyramine. Thy(6-4)Pyo is formed via the excited singlet state of Thy and it was not formed in the photosensitization by acetophenone (5).

To investigate the nature of the interactions between DNA and tyramine, we examined the effects of salts on the sensitization (Fig. 4). The sensitization was markedly diminished by increasing ionic strength. This is mostly explained by the electrostatic interaction of DNA with tyramine. The difference in the sensitization among tyramine, p-methoxyphenylethylamine, Lys-Tyr and tyrosine (Table 1) can probably be explained by the electrostatic interaction. Tyramine and p-methoxyphenylethylamine are the most effective sensitizers because they have a positive charge only. Lys-Tyr is the next because it has two positive and a negative charge. Tyrosine is the least effective among the above sensitizers because of a positive and a negative charge at neutral pH. Similar results are reported for the sensitization by acetophenone. The sensitization was enhanced when the acetophenone was substituted by a positively charged group (13). Lys-Tyr-Lys is further less effective probably because of a steric hindrance.

It was a remarkable point of our results that the sensitization was specific for native DNA (Table 1). The sensitization was six times larger for native DNA than for denatured DNA. In the case of the sensitization by acetophenone, on the other hand, the sensitized formation of Thy dimers was enhanced in denatured DNA compared with that in native DNA (14). In this case, the interactions are probably collision-like. In contrast with this, the interactions in the sensitization by tyramine seem somewhat different, i.e. tyramine in its excited triplet state is considered to form, at least in a moment of energy transfer to DNA bases, a transient complex which is specific for native DNA. To check the contribution of the electrostatic interaction or the formation of hydrogen bonding to the interactions, we examined the dependency of the sensitization on the change of the molecular structure of the sensitizer (Table 1). From the comparison in the efficiency of the sensitization between tyramine and p-ethylphenol, it is obvious that the positive charge at the ethylamino group of tyramine contributes greatly to the sensi-
tization but not prerequisite for it. Since hydrogen bonding by OH group of tyrosine ring does not contribute to the sensitization, hydrophobic interactions might contribute to it. From these results, the above-mentioned transient complex might be compatible with either in-groove location and/or transient intercalation of the sensitizer.

The possibility of the photosensitizatized dimerization of Thy in DNA of cells in vivo is only speculative. However, our observation that even such peptides as Lys-Tyr and Lys-Tyr-Lys can sensitize the photodimerization of Thy in DNA supports the above possibility. The observation (15) that the action spectrum of the formation of Thy$\cdot$Thy in cultured HeLa cell had its peak at 280 nm is another support. The sensitization by chromosomal proteins, if possible, might be influenced profoundly by the structure of nucleosomes. The examination of the possibility would give us new informations of the structure.

Abbreviations used are: thymine, Thy; cytosine, Cyt; uracil, Ura; cyclobutane ring in cis-sin dipyrimidine,●; 6,4'-[pyrimidine-2'-one]-thymine, Thy(6-4)Pyo.

REFERENCES