Studies on the Photoreactions of Coumarins and Furocoumarins

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ABSTRACT. The mechanism of skin-sensitizing photoreactions of coumarins and furocoumarins are studied by spectroscopic, triplet quenching, and fluorescence techniques. The excited singlet mechanism is suggested for xanthotoxin-thymine/or DNA photoreactions from the results of triplet quenching studies utilizing β-carotene as a quencher.

INTRODUCTION

The nature of excited states of skin-sensitizing coumarins and furocoumarins has received a good deal of attention. On the basis of luminescence spectra and theoretical calculations, it has been proposed that addition of furocoumarins to pyrimidine bases, free or in DNA, results from an attack of the (π,π*) triplet excited state. This was supported by quenching effect of oxygen and paramagnetic ions on the photodynamic effect of furocoumarins. However, coumarins dimerize to form C4-cyclo-

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adduct from both excited singlet (and/or singlet exciplex) and triplet state. Furthermore, most of the stereospecific photocycloadditions between olefins are known to be originated from the excited singlet states of singlet exciplexes. It is, therefore, suspected that addition of furocoumarins to pyrimidine bases responsible for photosensitization may result from an attack of the (π,π*) singlet excited state rather than the triplet state. The further study on the mechanism of C4-photocycloaddition of furocoumarins to pyrimidine bases is carried out to clarify this possibility utilizing spectroscopy, quenching, and fluorescence techniques.
EXPERIMENTAL

Materials. Coumarin (Aldrich Chemical Company), thymine (Sigma Chemical Co.), xanthotoxin (Sigma Chemical Co.), DNA from herring sperm (Calbiochem), and β-carotene (Eastman Organic Chemicals) were used as received without further purification. All solvents were redistilled prior to use.

Methods. The UV spectra were recorded on a Cary 14 spectrophotometer. The reaction was followed by measuring the change of absorbance at 266 nm and by studying thin layer chromatograms on silica gel G (Merck) plates. A Rayonet photochemical reactor Type RPR-100 (The Southern New England Ultraviolet Company) was used for the light sources. The light intensity was $4 \times 10^{-3}$ and $3 \times 10^{10}$ quanta/ml/min at 300 and 350 nm, respectively. Various ratios of concentrations between furocoumarins ($10^{-2}$~$10^{-5} M$) and pyrimidine bases in water, methanol, ethanol, and aqueous frozen solutions were irradiated at 300 or 350 nm.

Aqueous solutions of nucleic acid (0.01 %) containing 20 mM NaCl were prepared and ethanolic solution of xanthotoxin was added making the concentration of xanthotoxin to be 20 μg/ml. The final ethanol content was less than 1.5 % (solution A). After the addition of xanthotoxin, the solution was shaken for an hour at room temperature and filtered. The solution was irradiated at 350 nm in the presence or absence of β-carotene (dioxane solution) for 1.5 hours (at 15°C). To the irradiated solution, solid NaCl was added to make the final concentration to be 5 %, and ethanol was added to precipitate nucleic acid. The precipitated nucleic acid was separated by centrifugation (5,000 rpm for 25 minutes), washed with 80 % ethyl alcohol and dissolved in distilled water (solution B). The reaction was followed by measuring the absorbance of solution A and B. Fluorescence quenching was monitored by recording the fluorescence spectra of furocoumarin-pyrimidine base solutions in various concentration ratio on an Aminco-Bowman spectrophotofluorometer.

RESULTS and DISCUSSIONS

The ground state complex formation between furocoumarins and pyrimidine bases was studied in aqueous solutions. No complex formation is apparent since there is no change in the UV-VIS spectra when the concentration ratio of coumarin or xanthotoxin and pyrimidine bases, thymine, uracil, and cytosine, is varied. The ground state complex formation, therefore, is not involved in the $C_4$-photocycloaddition of furocoumarins to thymine. The $C_4$-photodimerization of coumarin and $C_4$-photocycloaddition of coumarin to thymine were studied in aqueous solution at room temperature and at the frozen state. The various concentration ratios of coumarin to thymine (1 : 1, 1 : 10, 1 : 100) are used and the results are summarized in Table 1 and 2. No change in absorbance was observed when the aqueous solution of coumarin and thymine (concentration ratio of 1 : 10) was irradiated at 350 nm for 1.5~3 hours at the frozen state indicating no reaction between coumarin and thymine.

![Non-fluorescent](image1.png)

$C_4$-Photoproduc between xanthotoxin and thymine.
and thymine under the condition given. From the results shown in Table 1 and 2, it is clear that coumarin undergoes C4-photodimerization as reported previously but does not react with thymine to give C4-photocycloaddition product contrary to the theoretical prediction. When xanthotoxin is irradiated with thymine or DNA in aqueous solution, several products are formed as reported by Musajo and Rodighiero12. These reactions were thought to undergo via excited triplet state, of xanthotoxin. The quenching of this triplet excited xanthotoxin in the presence of thymine or DNA is attempted with β-carotene as a quencher. The progress of photoreactions were monitored by measuring the change the change of absorbance in the UV-VIS spectra and the results are shown in Fig. 1 and 2 for thymine and DNA solution, respectively. From the spectra, it is clear that β-carotene does not quench the photoreaction of xanthotoxin-thymine/or DNA solutions contrary to our expectation for the triplet mechanism. The triplet energy

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Solution</th>
<th>water</th>
<th>ethanol</th>
<th>methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 nm</td>
<td>C&amp;T C</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>350 nm</td>
<td>C&amp;T C</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 1. Photochemical reactions of coumarin, coumarin thymine solutions at room temperature.

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>C&amp;T</th>
<th>C</th>
<th>C</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 nm</td>
<td>C</td>
<td>C</td>
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<td>C</td>
</tr>
<tr>
<td>350 nm</td>
<td>C</td>
<td>C</td>
<td>C</td>
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</tbody>
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Table 2. Photochemical reactions of coumarin and thymine in aqueous solutions irradiated at 360 nm for 72 hours at room temperature.

<table>
<thead>
<tr>
<th>Photoproduct</th>
<th>Counarin/Thymine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/1</td>
</tr>
<tr>
<td>C4-Coumarin dimer</td>
<td>1/10</td>
</tr>
<tr>
<td>C4-Cycloadduct</td>
<td>1/100</td>
</tr>
</tbody>
</table>

Fig. 1. Spectral changes on irradiation of xanthotoxin-thymine (1 : 10) in water; ○: prior to irradiation, no circles; after irradiation.

Fig. 2. Spectral changes on irradiation of xanthotoxin-DNA in water; ○: prior to irradiation, no circles; after irradiation.
of β-carotene is sufficiently low compared to that of furcocumarins \((55\sim 62 \text{ kcal/mole})^{13}\) since β-carotene even quenches singlet oxygen \((37 \text{ kcal/mole})\) through electronic energy transfer\(^{14}\). Furthermore, the phosphorescence lifetime of psoralen is relatively long \((9.66 \text{ sec})\) \(^{22}\) and the triplet state of xanthotoxin is expected to be quenched very efficiently by low energy triplet energy acceptors like β-carotene and molecular oxygen. This quenching study with β-carotene and that of Bevilaqua and Bordin with molecular oxygen and paramagnetic ions suggest that the photo reactions of xanthotoxin with thymine or DNA result probably from a shorter-lived singlet excited state than the triplet state of the xanthotoxin. This mechanism is also supported by the observation of very low intersystem crossing yields for coumarins \((\Phi_{isc} = 6 \times 10^{-3} \text{ in EtOAc} \text{ and } 8.8 \times 10^{-3} \text{ in acetonitrile for coumarin})\). Almost all of the excited coumarin molecules decay from the excited singlet states before crossing to the triplet state.

If the reactive transient is the excited singlet state, the xanthotoxin fluorescence is expected to be quenched by pyrimidine bases in high concentration. The quenching of xanthotoxin fluorescence by thymine and 1,3-dimethyluracil was tested by monitoring the change of fluorescence intensity versus pyrimidine base concentration in aqueous solution at room temperature. However, no quenching was observed in the range of \(0.025 \sim 0.4 \text{ M} \) pyrimidine base concentrations. This is probably due to the short lifetime of xanthotoxin excited singlet state \((1 \times 10^{-11} \text{ sec})\). Thus the fluorescence quenching studies neither prove nor disprove the singlet mechanism and further study is required to elucidate the mechanism of photoconcentration reactions of furcocumarins.

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REFERENCES