FLUORESCENCE QUENCHING OF DNA BY CARCINOGENIC QUINOLINES*1

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As a part of studies collecting fundamental informations on the intermolecular interaction between quinoline series carcinogenic compounds and biopolymers, comparative examinations were made on the quenching effect of 4-nitroquinoline 1-oxide and related carcinogens on the fluorescence of DNA. It was found that the fluorescence of DNA in acid solution decreases by the presence of quinolines. The degree of this quenching effect did not seem to parallel their biological activity. It was proved that the quenching of DNA fluorescence by quinolines is worked through purine moieties of the macromolecule.

Materials and Methods
Materials The source and purity of calf-thymus DNA, deoxyadenosine, and deoxyguanosine were the same as those described in an earlier work. All the quinoline derivatives used in the present work were also the same as those used in a previous work.

Solutions For the preparation of acid solution of DNA, calf-thymus DNA was dissolved in a mixture of 0.017M NaH$_2$PO$_4$ and 0.033M Na$_2$HPO$_4$, and this neutral solution was adjusted to pH 3.5 with conc. HCl. Absorption intensity of DNA in this solution at 259nm was 5% larger than that in the neutral solution. When nucleosides were used, a mixture (pH 3.5) of 0.01M Na$_2$HPO$_4$ and 0.015M citric acid was used as a solvent. All the solutions were shielded from light before measurement of spectra.

Spectrofluorometry Fluorescence spectra were recorded on a Shimadzu Model GF-16 spectrofluorophotometer. Changes in sensitivity of the photomultiplier according to the wavelength were not corrected. Accuracy of the fluorescence intensity was within the limit of ±1%. All the measurements were made at 25°C ± 0.1°C.

Spectrophotometry Absorption spectra were measured on a Hitachi Model EPS-3T recording spectrophotometer.

Results
Fluorescence Spectra of DNA and Purine Nucleosides
Fluorescence spectra of DNA solution at pH 3.5, excited at various wavelengths of 240 to 270 nm, are shown in Fig. 1. These spectra have a fluorescence maximum ($F_{\text{max}}$) at 355 nm and the shape of spectra was independent of the exciting wavelengths. Fluorescence was not observed in exciting light above 310 nm where DNA does not absorb the exciting light.

In the case of deoxyadenosine and deoxyguanosine in solution of pH 3.5, fluorescence spectra obtained by excitation at the wavelengths not affected by stray light, 259 nm, was used as the excitation wavelength.

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In later experiments on fluorescence quenching of DNA, the wavelength not affected by stray light, 259 nm, was used as the excitation wavelength.
position by fluorescence quenching experiment was proved by measuring the absorption spectra of solutions before and after the experiment.)

Fig. 3 (A, B) gives fluorescence spectra of deoxyadenosine and deoxyguanosine in the presence of quinolines. As will be clear from these graphs, the fluorescence of purine deoxyribonucleosides showed decreased intensity by the presence of the quinolines, without deformation of the spectral shape.

Under the present experimental condition, no change was produced in the visible region of absorption spectrum on mixing DNA or purine nucleosides with the quinolines.

Table II shows the decrease in fluorescence intensity of DNA and deoxyribonucleosides by

![Fig. 1. Fluorescence spectra of DNA excited at various wavelengths (pH 3.5)](image)

Excitation wavelengths: curve 1, 270 nm; curve 2, 260 nm; curve 3, 250 nm; curve 4, 240 nm.
Concentration of DNA: 0.05 mg/ml.
Inserted graph shows the excitation spectrum of DNA at pH 3.5.

![Fig. 2. Fluorescence quenching of DNA by quinolines at pH 3.5](image)

Curves: 1, DNA; 2, DNA plus 4-nitroquinoline (V); 3, DNA plus 2-methyl-4-nitroquinoline 1-oxide (IV); 4, DNA plus 4-nitroquinoline 1-oxide (III); 5, DNA plus 6-chloro-4-nitroquinoline 1-oxide (II); 6, DNA plus 4,6-dinitroquinoline 1-oxide (I).
Concentration: DNA, 0.05 mg/ml; quinoline, 2.5 x10^-5 M.
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quinolines, expressed by percentage quenching. These values have been corrected with inner-filter effect of the quencher at the excitation wavelength according to equation (1).

\[
\text{Quenching (\%) =} \frac{1 - F/F_0}{p} \times 100 \quad (1)
\]

where \( F \) and \( F_0 \) are the fluorescence intensity in the presence and absence of the quencher, respectively, and \( p \) is a correction coefficient expressed by equation (2).

\[
p = \frac{1 - e^{-\frac{\epsilon_F}{\epsilon_Q} F}}{\epsilon_Q + \epsilon_F} \quad (2)
\]

where \([F]\) and \([Q]\) are the concentration of fluorescer and quencher, respectively, and \(\epsilon_F\) and \(\epsilon_Q\) are the molecular extinction coefficients of fluorescer and quencher, respectively (cf. Ref. 6).

As will be clear from Table II, the quenching effect of quinolines on the fluorescence spectrum of DNA decreased in the order of 4,6-dinitroquinoline 1-oxide (I) > 6-chloro-4-nitroquinoline 1-oxide (II) > 4-nitroquinoline 1-oxide (III) > 2-methyl-4-nitroquinoline 1-oxide (IV) > 4-nitroquinoline (V). It is also clear from Table II that the quenching effect of the quinolines on the fluorescence of both nucleosides decreased in the same order as in the case of their effect on the macromolecule. It is notable that in all the quinoline systems, larger fluorescence quenching effect was seen for deoxyadenosine than for deoxyguanosine.

![Fig. 3. Fluorescence quenching of deoxyadenosine (A) and deoxyguanosine (B) by quinolines at pH 3.5](image)

Curves (number of quinolines as in Fig. 2): 1, nucleoside alone; 2, \( V+nucleoside \); 3, \( IV+nucleoside \); 4, \( III+nucleoside \); 5, \( II+nucleoside \); 6, \( I+nucleoside \).

Table II. Fluorescence Quenching of DNA and Purine Nucleosides by Quinolines

<table>
<thead>
<tr>
<th>Quencher</th>
<th>Compd. No.</th>
<th>DNA</th>
<th>Quenching (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Deoxyadenosine</td>
</tr>
<tr>
<td>4,6-Dinitroquinoline 1-oxide</td>
<td>I</td>
<td>25</td>
<td>31</td>
</tr>
<tr>
<td>6-Chloro-4-nitroquinoline 1-oxide</td>
<td>II</td>
<td>21</td>
<td>27</td>
</tr>
<tr>
<td>4-Nitroquinoline 1-oxide</td>
<td>III</td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td>2-Methyl-4-nitroquinoline 1-oxide</td>
<td>IV</td>
<td>17</td>
<td>22</td>
</tr>
<tr>
<td>4-Nitroquinoline</td>
<td>V</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>
Discussion

In the present series of work, we examined the quenching effect of carcinogenic quinolines on the fluorescence of DNA. From the spectral characteristics stated above, there is no doubt that the fluorescence spectrum measured originates in DNA itself. It is well known that the fluorescence of nucleic acids is due to purine bases, and pyrimidines do not contribute at all to the emission.\(^1\)

The present results showed that the fluorescence of DNA is quenched more or less by carcinogenic quinolines. The order of the quenching effect of quinolines against the fluorescence of DNA was the same as that observed at the nucleoside level. This fact clearly indicates that the quenching effect of the quinolines on the fluorescence of DNA is worked through interaction of the quenchers with purine moieties of the macromolecule.

The fact that no change was produced in the visible region of absorption spectrum on mixing DNA or purine nucleosides with quinolines indicates that there has been virtually no charge transfer interaction between the componental molecules under these experimental conditions in ground state (a charge transfer band appears in the visible region only when the concentration of the component is much higher than that in the present work\(^8\)). The order of the quenching effect of quinolines against the fluorescence of purine nucleosides was in parallel with the order of the energy level of the lowest-empty orbital of the quinolines.\(^7\) This fact suggests that the electronic state of the \(\pi\)-system of the quencher is in some way related to the quenching phenomenon. However, as mentioned above, the extent of fluorescence quenching was larger in the deoxyadenosine system than in the deoxyguanosine system, contrary to the fact that the energy level of the highest-occupied orbital of adenine is higher than that of guanine.\(^9\) It is considered, accordingly, that the mechanism of the interaction between the fluorescers and quenchers in the present work will not be a simple one.

The carcinogenic activity of the quinoline derivatives used here is reported to be as follows: I is weakly carcinogenic,\(^12\) II is a potent carcinogen and its activity is stronger than that of III,\(^13\) while the activity of IV is about the same as that of III.\(^10\) V is highly carcinogenic.\(^6\) As far as II, III, and IV are concerned, there seems to be a correspondence between the degree of quenching effect on the fluorescence of DNA and the carcinogenic activity. In the case of I and V, however, the observed degree of quenching effect does not parallel their biological activity.

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References

5) Nakahara, W., Arzneimittel-Forsch., 14, 842 (1964).
8) Okano, T., Isobe, A., Matsumoto, H., Gann, 63, 427 (1972).
9) Okano, T., Maenosono, J., Kano, T., Onoda, I., Gann, 64, 227 (1973).