Photoreactivity of Chlorpromazine with Native DNA in an Aqueous Solution

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Near-UV irradiation of a mixture of chlorpromazine and native DNA caused irreversible binding of the drug or its photoproduct(s) to DNA and double strand break of DNA. When the irradiation was performed in a reaction mixture with a low salt concentration, much more photobinding occurred. Accompanying these effects, the maximum hyperchromicity of DNA at a high temperature was decreased. This can be explained by either a partial denaturation or an inhibition of melting by a formation of complex between double helical DNA and a promazine polymer.

INTRODUCTION

Near-UV radiation which is a component of sun light is the most ubiquitous mutagen in the environmental factors for human being. Particularly, it induces the large deleterious effects on biological systems when some kind of photosensitizers is present. The photosensitizers involve naturally occurring compounds such as porphyrins, aflatoxin, psoralens and phaeophorbide, and synthetic compounds which are used as medicines, cosmetics or sometimes food additives. Of medicines, phenothiazines, thiazides and tetracyclines are known as the example of phototoxic or photoallergic drugs. In spite of a number of investigations on the synergistic effect of near-UV light and these photosensitizers, the action mechanisms on the molecular basis are still unclear except for the action of psoralens.

Chlorpromazine (CPZ), a psychotropic drug, sometimes elicits contact dermatitis and cataracts. With respect to the photosensitization on cells, membrane proteins and phospholipids are feasible to be the target materials because CPZ shows a high affinity to cellular membranes. On the other hand, DNA can also be a target because photomutagenesis has been detected in Chinese hamster cells and Salmonella typhimurium. Jose discussed the correlation between cataracts and DNA damage. We have studied the photosensitized action of CPZ on E. coli and bacteriophages.

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to know nature of damage produced by the photosensitization. Single strand break of DNA was detected in the treated phage lambda\(^{19}\). Similarly, single strand break of DNA was observed in adeno virus \(^{5}\) and in human fibroblasts (B. Ljunggren et al., \textit{J. Invest. Dermatol.}, in press). The spectrofluorometric analysis\(^{15,16}\) and the radioactive labeling technique (I. E. Kochevar et al., 8th Intern. Congress of Photobiol., 1980) demonstrated that, in an aqueous solution, CPZ reacts preferentially with single-stranded DNA upon near-UV irradiation.

In the present study, near-UV effects on the structure of native DNA in an aqueous solution containing CPZ were examined to obtain direct evidence for DNA damage by CPZ-photosensitization. This report describes the results which reveal the irreversible binding of CPZ or its photoproduct(s) on DNA, the double strand break of DNA and the repression of hyperchromicity of DNA upon heating.

**MATERIALS AND METHODS**

\textit{Materials}

Chlorpromazine hydrochloride [2-chloro-N-(3-dimethylaminopropyl) phenothiazine hydrochloride] (see Fig. 1) was supplied by the Shionogi and Co. and the purity was confirmed by thin layer chromatography on Kiesel gel 60 F\(_{254}\) plates (Merck) developed with ethylacetate/acetic acid/H\(_2\)O (8/3/3). Calf thymus DNA, type I, was purchased from the Sigma Chemical Co. Lambda DNA was prepared from phage \(\lambda\) by the phenol extraction\(^{17}\). Preparation method of the phage suspension was the same as described previously\(^{13}\).

Near-UV irradiation

A fluorescent tube, Toshiba FL 20 s BLB (Torex), was used as a source of black light. The luminosity spectrum was reported in our previous paper\(^{12}\). The irradiation was performed at a fluence rate of 7.5 J/m\(^2\)/s which was measured with a UV-radiometer (Toshiba, UVR-365) calibrated by potassium ferrioxalate actinometry. DNA was dissolved in 250-fold diluted standard saline-citrate (1/250\(\times\)SSC) unless otherwise stated. A small volume of the stock solution of CPZ was added to a solution of DNA to make a final concentration of CPZ as 100 \(\mu\)g/ml (2.8 \(\times\)10\(^{-4}\) M). The mixture (5 ml) was irradiated with black light in a glass Petri dish (inner diameter, 9 cm) covered with a glass lid under conditions equilibrated with air. The concentrations of calf thymus DNA and lambda DNA were 10.3\(\times\)10\(^{-6}\) and 4.5\(\times\)10\(^{-5}\) M, respectively. When the samples for electron microscopy were prepared, a mixture (0.5 ml) containing 5.8\(\times\)10\(^{-6}\) M \(\mu\)g lambda DNA and 100 \(\mu\)g/ml CPZ (in 1/25\(\times\)SSC) was irradiated in a glass Petri dish (inner diameter, 3 cm). In every cases, the DNA samples were dialyzed extensively to remove free CPZ and free photoproducts against 1/25\(\times\)SSC after the irradiation. To control
samples was also added CPZ, and it was then dialyzed.

**Measurements of absorption spectra and melting curves**

The dialyzed solutions were supplied to measurements of the absorption spectra and the melting curves. Measurements were performed with a Hitachi spectrophotometer, Model 124. Monitoring $A_{268}$, the sample solution was heated at a rate of 3°C/min and chilled at a rate of 1°C/min with a programmed heating device (Komatsu Electronic, Model KPC-3 and Model SPR).

**Electron microscopy**

Specimens of lambda DNA were made by the Kleinschmidt spreading technique modified by Davis et al., and photographed with a JEOL electron microscope, JEM 100c, to measure molecular lengths of the double-stranded DNA.

**RESULTS AND DISCUSSION**

**Dark interaction**

Change in the absorption spectrum of CPZ was observed when calf thymus DNA was added. As shown in Fig. 2, the absorption peak was lowered and shifted to long

![Fig. 2. Change of absorption spectrum of CPZ by addition of DNA. Difference spectra were measured when DNA was added. Concentrations: CPZ, 50 µg/ml; calf thymus DNA, 0, 5.2×10^{-5} (1) and 20.6×10^{-5} M_p (2) in 1/25×SSC. The concentration of DNA was calculated from $A_{280}$ assuming that $s_p=6,600$.](image)
wavelengths with increasing concentrations of DNA. At an extremely high concentration, e.g., $1.68 \times 10^{-8} \text{M}_p$ of the DNA, the absorption peak was shifted to 330 nm (data not shown). The isosbestic point was observed at 325 nm. According to the X-ray diffraction data, the molecular plane of CPZ is not flat but folded about the S-N axis with an angle of 139.4°. However, the weak binding with partial intercalation of one of phenyl rings between nucleic acid base pairs can be considered as proposed by Kantesaria and Marfey. The observed spectral modification is a reflection of the interaction between CPZ and nucleic acid bases.

**Photobinding**

After the dialysis of an irradiated solution of calf thymus DNA-plus-CPZ, the absorption spectrum was measured. The result indicates the increase in the absorbance in a far-UV range and the appearance of a shoulder in a near-UV range (Fig. 3). The

![Absorption spectra](image)

*Fig. 3.* Absorption spectra after the irradiation with black light and the subsequent dialysis. Concentrations when irradiated: calf thymus DNA, $10.3 \times 10^{-8} \text{M}_p$; CPZ, 100 μg/ml in 1/250×SSC. Irradiation times are indicated in the figure by min.
spectra of the net increase in absorbance were drawn assuming that absorbance by the DNA remains unaltered. They did not coincide with the absorption spectrum of free CPZ (Fig. 4). The increase suggests the irreversible binding of CPZ or a certain kind of its photoproduct(s) to DNA. The same modification was observed in the photoreaction with lambda DNA (data not shown).

Figure 5 implies that the binding amounts were increased with decreasing salt concentrations when a fixed fluence was given. This may result from the contribution of ionic interaction between CPZ cations and DNA phosphate on the dark interaction prior to the photoreaction.

Abnormality in the melting curve

The thermal denaturation and renaturation curves of the treated DNA were drawn by monitoring the absorbance at 258 nm. The observed curves are shown in Fig. 6(a). The measured value of $A_{258}$ includes not only the absorbance by DNA but also the absorbance by the irreversibly bound CPZ or its photoproduct(s). Therefore, assuming that the value of $(A_{t_{irrad}} - A_{t_{unirrad}})$, namely, the absorbance by the bound CPZ or its bound photoproduct(s) at $t^\circ$C, is independent on temperature, a value of the hyperchromicity ($H$) was corrected as follows:

$$H_{corrected} = \frac{A_{t_{irrad}} - (A_{t_{irrad}}^{25} - A_{t_{unirrad}}^{25})}{A_{t_{unirrad}}^{25}}$$
where $A_{t}^{irrad}$ and $A_{25}^{irrad}$ are the observed absorbance of an irradiated sample at $t^\circ C$ and $25^\circ C$, respectively, and $A_{25}^{unirrad}$ is the absorbance of DNA alone at $25^\circ C$. The corrected melting curves are shown in Fig. 6(b). Similarly, the corrected melting curves of the treated DNA of phage lambda are shown in Fig. 7. When subjected to the photosensitized reaction, the maximum hyperchromicity of DNA at a high temperature was decreased but the temperature of melting was not altered. This abnormal feature in the melting profiles can be explained by either a partial denaturation of DNA or a formation of firmly bound complex between double helical DNA and cationic promazine polymer photoproducts. We have no direct evidence yet to determine which is operative. In spite that a promazine polymer was reported to be a photoproduct of CPZ by an anaerobic irradiation, the latter possibility can not be excluded, because an aerobic irradiation also formed a photoproduct which remained at the origin on a Kiesel gel F254 plate (unpublished finding). Interstrand crosslinking as in the case of psoralen-photosensitization can not be predicted from the molecular structure of CPZ. In fact, no efficient renaturability was observed during the slow chilling process.
Fig. 6. Observed heating-and-chilling curves (a) and the corrected heating curves (b) of calf thymus DNA after the photoreaction. Samples are the same as those shown in the caption of Fig. 3. Irradiation time: 0 (○), 1 (△) and 2 min (×).

Strand break of DNA

Apparent molecular size of lambda DNA was measured by electron microscopy. The results are summarized in Table 1. The observed size of the unirradiated DNA was about 1/8 of the intact size due to the breakage by a shearing force during the preparation. Nevertheless, double strand scission of the DNA by the photoreaction is evident.

In conclusion, it was confirmed that DNA is injured by near-UV irradiation in the presence of CPZ. The treatment causes the irreversible photobinding of CPZ or its photoproduct(s) to DNA and strand breakage of DNA. Accompanying the photobinding and the strand breakage, an increase in the hyperchromicity of DNA upon heating is repressed.

Although smaller amounts of CPZ or its photoproduct(s) bind to double-stranded DNA by irradiation compared with the photobinding to single-stranded DNA, it is possible that DNA is partially denatured by the irreversible binding of CPZ or its
photoproduct(s). As a result of the partial denaturation, the photobinding is accelerated around the sites of the preceding binding. Therefore the irreversible binding and the partial denaturation could proceed cooperatively.

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