INFLUENCE OF PRIOR COMPLEX FORMATION ON THE PHOTOADDITION OF CHLORPROMAZINE TO CALF THYMUS DEOXYRIBONUCLEIC ACID†

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Summary

Chlorpromazine (CPZ), 2-chloro-*N*-(3-dimethylaminopropyl) phenothiazine, causes cutaneous photosensitivity in man. The photoaddition of CPZ to deoxyribonucleic acid (DNA) may be an important mechanism for the phototoxicity. We have investigated the complexes formed between CPZ and calf thymus DNA prior to irradiation, related their formation to the photoaddition of CPZ to DNA and initiated studies to identify the photoadducts. In the presence of high concentrations of double-stranded DNA, the CPZ absorption maximum shifted from 305 to 340 nm with an isosbestic point at 323 nm. The CPZ fluorescence at 460 nm was quenched a maximum of 90%. The excitation and emission spectra for the unquenchable fluorescence are the same as those for free CPZ. These results together with those from flow dichroism measurements indicated that CPZ formed two complexes with double-stranded DNA. One complex involves intercalation, is non-fluorescent and absorbs at 345 nm. The second complex absorbs at 310 nm, fluoresces at 460 nm and has the phenothiazine ring parallel to the DNA axis. Non-covalent binding of CPZ to heat-denatured DNA did not shift the CPZ absorption spectrum but quenched 65% of the CPZ fluorescence. One complex between CPZ and denatured DNA will account for these results. CPZ photolysis was inhibited compared with that of free CPZ by binding to double-stranded DNA (more than 98%) or denatured DNA (65%). CPZ photoadded ten times more efficiently to denatured DNA than to double-stranded DNA. These results indicate that CPZ photolysis and photoaddition are quenched in the intercalation complex. The photoaddition to double-stranded DNA does not result from intercalated CPZ because the action spectrum maximized at 310 nm rather than at 340 nm.

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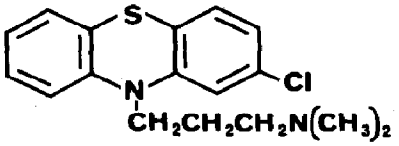
Photochemistry in skin has both deleterious and beneficial aspects. The most common effects of light on skin are sunburn, tanning and formation of pre-vitamin D. Deleterious effects of light on skin which are currently being investigated include phototoxic reactions to chemicals and UV carcinogenesis. Phototoxicity is an adverse response of skin to a combination of exposures to a chemical and light, usually of 300 - 400 nm. The response of the skin may look like a severe sunburn or may involve itching, tingling, pain or other symptoms.

Photochemistry *in vivo* is usually more complex than the photoreactions of the same molecule in solution because of the numerous reaction partners available and the non-homogeneous medium. The compound may associate with specific molecules in the cell (proteins, deoxyribonucleic acid (DNA)) or reside in particular structures (membranes, lysozomes). A major challenge in studying mechanisms for phototoxicity responses is to correlate the results of *in vitro* photochemical studies with those of *in vivo* photochemistry.

We have been studying the photoreactions of chlorpromazine, 2-chloro-*N*-(3-dimethylaminopropyl) phenothiazine (CPZ), a well-known cutaneous photosensitizer [1]:



CPZ is frequently prescribed as an antipsychotic drug. It forms photoadducts with proteins [2] and DNA [3], photosensitizes lipid oxidation [4] and chain cleavage of DNA [5] and forms stable photoproducts which act like detergents on biological membranes [6, 7]. In this paper we focus on our results concerning the photoaddition of CPZ to DNA, which may be the basis for the photomutagenesis and phototoxicity of CPZ.

CPZ is a water-soluble phenothiazine derivative with absorption maxima at 255 and 310 nm. The photoaddition of CPZ to DNA was studied initially by Kahn and Davies [3] who made the interesting observation that the photoadduct yield was higher between CPZ and denatured (single-stranded) DNA than it was between CPZ and native (double-stranded) DNA. Their results contrasted with what was known about psoralens, another type of phototoxic compound. The photoaddition of psoralens to DNA appears to be the basis for a very effective phototherapy for psoriasis [8, 9]. Psoralens (planar furocoumarins) intercalate between base pairs in double-stranded DNA. Intercalation is a prerequisite for subsequent photoaddition which binds the psoralen via cyclobutane rings mainly to thymines.

We decided to investigate the influence of the complex(es) formed between CPZ and DNA on the photoreactions of CPZ with DNA [10]. Absorption and fluorescence measurements were used to detect the species present in solution when DNA (native or denatured) was added to CPZ.

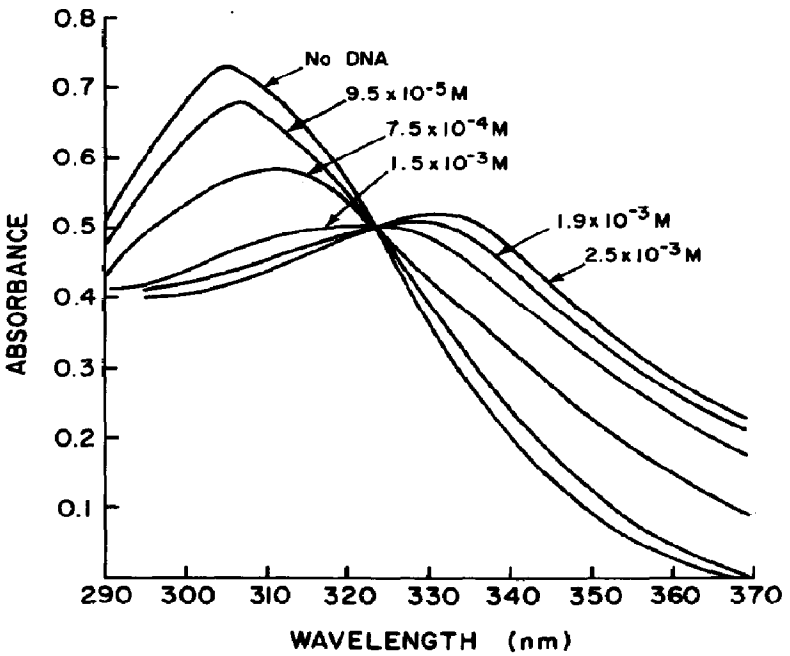


Fig. 1. Difference spectra of 0.15 mM CPZ in unbuffered solution in the presence of double-stranded calf thymus DNA. DNA was added to both the reference and the sample cuvettes to give the concentrations indicated.

The CPZ absorption maximum red shifted about 30 nm (from 310 to 340 nm) in the presence of native calf thymus DNA. The maximum shift was obtained at a ratio of base pair to CPZ of greater than 8 (Fig. 1). The red shift is accompanied by a decrease in the extinction coefficient (from 5000 to 3500 M^{-1} cm^{-1}). An isosbestic point occurred at 323 nm. A shift in the DNA absorption at 260 nm had been reported in the presence of CPZ but was difficult to interpret because the CPZ absorption maximum at 250 nm may also shift on binding to DNA [11]. Single-stranded DNA (heat denatured) did not shift the CPZ absorption maximum although about 10% hypochromicity was observed. These results indicate that CPZ binds to double-stranded DNA in the ground state to form a complex absorbing at 340 nm. Dialysis experiments indicated that the binding was reversible.

The binding of CPZ to native and denatured calf thymus DNA was also followed by monitoring the quenching of the CPZ fluorescence at 460 nm. Fluorescence was excited at 323 nm, the isosbestic point of the absorption spectrum of mixtures of CPZ and native DNA. Addition of native DNA quenched the CPZ fluorescence a maximum of about 90% (Fig. 2). No new emission was observed at longer wavelengths. The fluorescence emission and excitation maxima of the unquenchable fluorescence were the same as those for free CPZ. These data indicate that two complexes are formed between CPZ and double-stranded DNA; one non-fluorescent complex absorbs at 340 nm and another absorbs at 310 nm and fluoresces at 460 nm. It is necessary to postulate the second complex

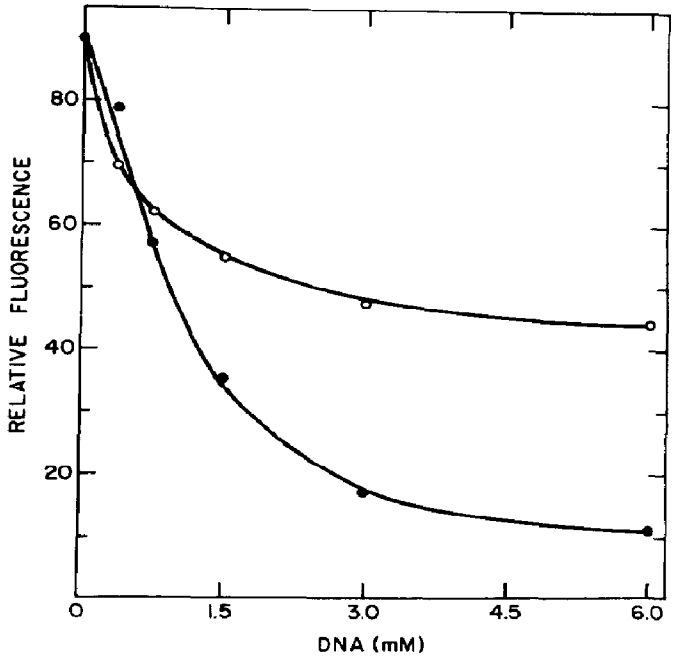


Fig. 2. Fluorescence of CPZ excited at 323 nm and emitting at 460 nm in the presence of various concentrations of native (●) and denatured (○) calf thymus DNA ([CPZ] = 0.05 mM; excitation slit, 4 nm). (By courtesy of Elsevier Scientific Publishers Ireland Ltd.)

because the fluorescence which asymptotically approaches about 10% at high DNA concentrations has a fluorescence excitation maximum at 310 nm rather than at the absorption maximum (340 nm) of the first complex.

Denatured DNA also quenched the CPZ fluorescence; the maximum quenching was 65% at high DNA concentrations (Fig. 2). Some of the quenching may be due to CPZ bound to remaining double-stranded regions in the denatured DNA. However, this cannot represent a large portion of the CPZ molecules because the CPZ absorption spectrum excitation maximum was 310 nm. These data indicate that one complex is formed between CPZ and denatured DNA which has a quantum yield for fluorescence that is 65% of that of free CPZ.

Studies on the effects of Na^+ and Mg^{2+} on the formation of the complex absorbing at 340 nm indicated that CPZ partially or fully intercalates between base pairs. More detailed information about the complexes formed between native DNA and CPZ was obtained from flow dichroism studies. These studies were performed in collaboration with N. Geacintov at New York University. The dichroism spectrum is negative for the nucleic acid bases and for any molecule bound to DNA which lies parallel to the plane of the base pairs (provided that its transition moment is in the plane of the molecule). At high ratios of the DNA base pair to CPZ the dichroism spectrum is negative and resembles, but does not mirror, the absorption spectrum of the solution. The transition moment of the phenothiazine ring is along the

long axis of the molecule. Our results indicate that two complexes exist: one absorbing at 345 nm that involves intercalation of the phenothiazine ring between base pairs and another absorbing at 310 nm that is oriented with the phenothiazine ring parallel to the axis of the DNA helix.

The results of the absorption, fluorescence and flow dichroism measurements are summarized in the following scheme (where n-DNA denotes native DNA and dn-DNA denotes denatured DNA):

$$\text{complex } I_n \rightleftharpoons \text{CPZ} + \text{n-DNA} \rightleftharpoons \text{complex } II_n$$

(absorbance, 310 nm; fluorescence, 460 nm) (absorbance, 340 nm; fluorescence, 0)

$$\text{complex } I_{dn} \rightleftharpoons \text{CPZ} + \text{dn-DNA}$$

(absorbance, 310 nm; fluorescence, 460 nm)

In complex I_n, the phenothiazine ring is approximately parallel to the axis of the double helix and the complex fluoresces at 460 nm. In complex II_n the phenothiazine ring is flattened from its bent shape in solution [12] in order to intercalate between the base pairs and is non-fluorescent.

Binding of CPZ to denatured DNA to form complex I_{dn} cannot involve intercalation although "stacking" of the phenothiazine ring and nucleotide bases may occur. Evidence for ground state complexes between CPZ and purine nucleotides has been reported [11, 13]. This interaction may account for the quenching of CPZ fluorescence by denatured DNA.

The overall goal of our research now is to determine the relationship between these complexes and the photochemistry of CPZ with DNA. Complexing between CPZ and DNA affects the rate of CPZ photolysis. Solutions containing 0.075 mM CPZ and 1.0 mM DNA were irradiated at 323 nm (the isosbestic point) where the absorbance was 0.22. The photolysis of CPZ plotted according to first-order kinetics gave straight lines for CPZ alone and for CPZ in the presence of single- and double-stranded DNA (Fig. 3). The rate of CPZ photodecomposition was decreased by a factor of about 2.5 in the presence of denatured DNA compared with that for CPZ alone and was almost totally abolished in the presence of native DNA. The same CPZ photoproducts were formed in the presence and absence of DNA as detected by high performance liquid chromatography (HPLC). The percentage conversion was minimized (less than 20%) because the photoproducts of CPZ (promazine, chlorpromazine sulfoxide and others) absorb at 323 nm. These results indicated that prior complexing of CPZ to DNA "protected" it from photolysis; the complexes formed with native DNA almost totally quenched the photolysis. The degree of protection correlated with the quenching of CPZ fluorescence by native and denatured DNA.

We confirmed the previous result that CPZ photoaddition is more efficient with denatured DNA as the substrate than with native DNA (Fig. 4). ^{3}H(benzene ring)-labelled CPZ was irradiated in the presence of native

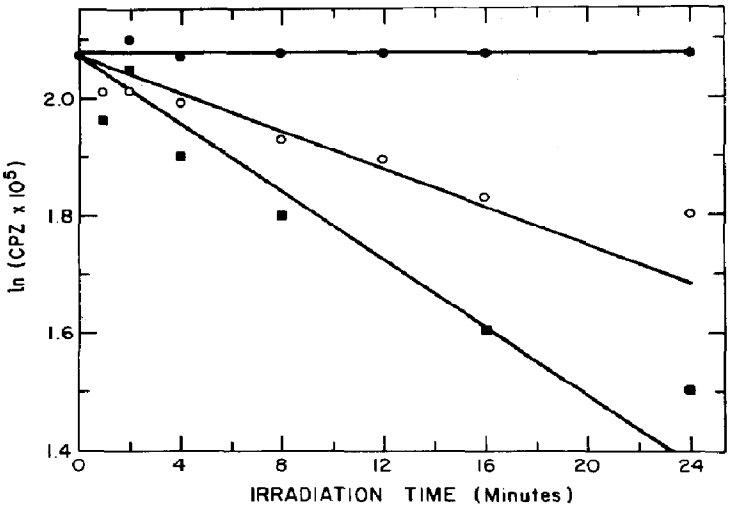


Fig. 3. Photolysis of 0.075 mM CPZ in aqueous solution ([CPZ] = 0.075 mM; irradiation at 323 ± 4 nm): ■, no DNA; ●, in the presence of 1.0 mM native calf thymus DNA; ○, in the presence of 1.0 mM heat-denatured DNA. (By courtesy of Elsevier Scientific Publishers Ireland Ltd.)

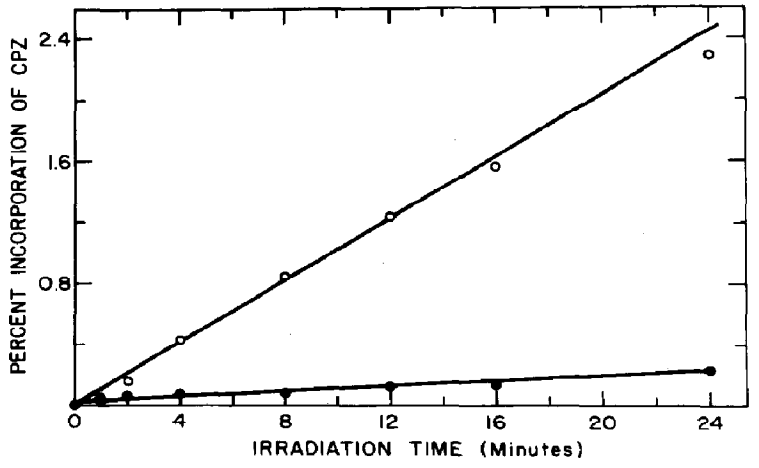


Fig. 4. Photoaddition of CPZ to native calf thymus DNA (●) and to denatured DNA (○) ([CPZ] = 0.075; [DNA] = 1.0 mM; irradiation at 323 ± 4 nm). (By courtesy of Elsevier Scientific Publishers Ireland Ltd.)

and denatured DNA and the radioactivity associated with the DNA after purification was determined. Under the same conditions used for the CPZ photolysis experiment, 2.3% of the ^{3}H CPZ label was associated with denatured DNA whereas only 0.22% was bound to native DNA. An action spectrum for the photoaddition of CPZ to native DNA maximized at 310 nm rather than at 345 nm, indicating that photolysis of intercalated DNA did not result in photoaddition. Our results show that about 10% of the CPZ which is photolyzed in the presence of denatured DNA becomes covalently bound and about 40% of the CPZ photolyzed can be accounted

for by the soluble photoproducts that are detected by HPLC. The rest of the CPZ photolyzed is probably accounted for by higher molecular weight photoproducts which do not elute under our HPLC conditions [7].

Photoaddition of CPZ to DNA probably results from dehalogenation because promazine does not photoadd to DNA under our conditions. In alcoholic solution triplet CPZ cleaves to form promazyl and chlorine radicals [14]. The mechanism of dehalogenation has not been established in aqueous solution. Radical addition to purines is expected to occur at C(2) or C(8). We are currently studying the photoaddition of CPZ to deoxyguanosine and guanosine-5′-monophosphate.

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