Photochemistry in Micelles as a Model for the *in vivo* Phototoxicity of Chlorpromazine

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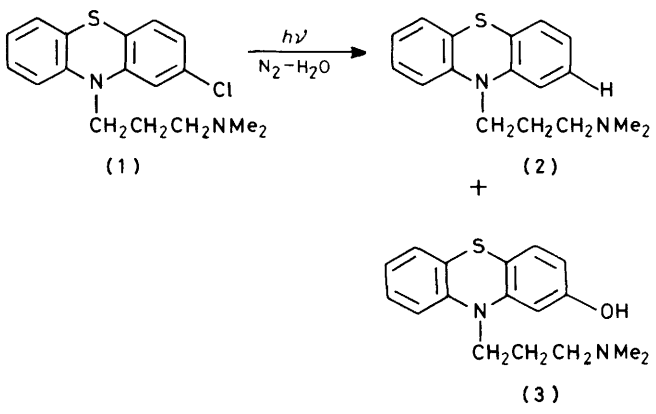
The photochemical reactivity of chlorpromazine has been studied in an oxygenated solution containing micelles. The photochemical dehalogenation reaction which had previously only been observed in oxygen-free solutions was found to predominate. The relevance of this photoreaction and the micelle as a model for the *in vivo* phototoxicity of chlorpromazine is discussed.

The wide use of chlorpromazine (CPZ) (1) in psychotherapy has led to the observation of light-induced side effects, such as exaggerated sensitivity of the skin to sunburn. A number of investigators have studied its photochemical behaviour to try and understand the cause of the undesired photobiological activity, and have established that the photochemistry of CPZ is complex. Huang and Sands [1] and later Hashiba [2] established that prolonged irradiation of CPZ in aerated water leads to at least a dozen products, most of which have not been identified. Later Huang and Sands [3] reported that in a *nitrogen*-saturated aqueous solution the photoreaction was very different, giving a number of products, including promazine (PRO) (2) and 2-hydroxypromazine (3). Grant and Greene,[4] Davies,[5] and Rosenthal [6] all found that in irradiation of CPZ in nitrogen-saturated alcohol solutions promazine and the 2-alkoxypromazine was formed. Davies [5] also noted that in oxygen-free propan-2-ol the photochemistry is much cleaner than it is under other conditions, and focused attention on the photochemical dehalogenation of CPZ *via* a radical pathway as the likely source of the light-induced cellular damage. A number of studies are consistent with this hypothesis, such as Nilsson's observation [7] that singlet oxygen seems not to be involved in CPZ-photosensitized erythrohemolysis, and Kochevar and Lamola's report [8] that the rate of red blood cell lysis is unaffected by the absence of oxygen during irradiation. However, the relevance of the photofragmentation of CPZ (to form dehalogenated products) to its phototoxicity has been questioned because when oxygen is present during irradiation in solution the predominant reaction is photo-oxidation of the CPZ. It has been further argued that oxygen-free solutions are poor models for cells and are of little value in clarifying the chemical changes that cause biological damage.

We were intrigued by evidence that CPZ self-associates into micelles [9] as well as associating with various biopolymers.[10] Since the photoreaction of chlorpromazine appears to be medium-dependent we felt that the photolysis of it in a micelle may provide a more suitable model for a ' typical ' biological environment than would a dilute aqueous solution. Our studies show that it may be possible for photochemistry that is *absent* in oxygenated aqueous-phase photolysis to actually be a contributor to *in vivo* phototoxicity.



(1) (2) + (3)

Experimental

Chlorpromazine hydrochloride (Sigma) was found to be of acceptable purity as received; purification by chromatography and crystallization gave material yielding identical results. The free base (prepared by basification of an aqueous solution with ammonium hydroxide and extraction into chloroform) gave the same results as the hydrochloride, apparently because of the buffering effect of the detergent (the pH of the two solutions were experimentally identical). Authentic promazine was similarly prepared from the hydrochloride (H. L. Moore) for spectroscopic and chromatographic comparison with the isolated photoproduct. Detergents used were Brij 58 (Sigma), sodium dodecyl sulphate (MCB), and hexadecyltrimethylammonium chloride (Eastman). 2,5-Dimethylfuran (Aldrich) and absolute ethyl alcohol (U.S. Industrial Chemicals) were used without further purification. Ethyl acetate (Aldrich spectrophotometric grade) was distilled through a 4 × 100 cm column packed with porcelain saddles. Chloroform (Aldrich spectrophotometric grade) was passed through a column of activated alumina (Fisher adsorption grade).

Solutions of chlorpromazine hydrochloride (*ca.* 1×10^{-3}M) in the detergent solutions were irradiated in quartz tubes in a Rayonet preparative photochemical reactor equipped with 350 nm lamps for times ranging from 2.5 to 40 min. Solutions for irradiation were purged either with oxygen or nitrogen for 15 min prior to and during irradiation. The concentration of chlorpromazine was kept well below the critical micelle concentration (c.m.c.) of 1.9×10^{-2}M but the concentration of the detergents was considerably higher than their c.m.c.: Sodium dodecyl sulphate at 3×10^{-3}M or higher, hexadecyltrimethylammonium chloride at 9.4×10^{-3}M, and Brij 58 at 1.3×10^{-2}M. The concentration of detergent was normally at least 50 times the molar concentration of chlorpromazine; with aggregation numbers of 50—100 and uniform distribution of CPZ about one molecule of CPZ per micelle would result, though this was not experimentally tested. Only very high concentrations of CPZ in micelles (detergent : CPZ ratio of 20 : 1) gave different results, with promazine no longer being observed as a product.

Isolation of photoproducts from Brij 58 solutions after irradiation was accomplished by diluting 25 ml to 250 ml with water, extraction with 3 × 75 ml of chloroform, and concentration on a rotary evaporator to 5 ml. These solutions were chromatographed using a Michel-Miller (Ace Glass) high performance low pressure liquid chromatography

Table. Yield of promazine from CPZ photolysis under various conditions

Solution	Atmosphere	Yield (%)
Water	Nitrogen	38
Water	Oxygen	0
Water–Brij 58	Nitrogen	55
Water–Brij 58	Oxygen	46
Water–SDS	Oxygen	38
Water–HTC	Oxygen	41
Water–Brij 58–DMFU	Nitrogen	56
Water–Brij 58–DMFU	Oxygen	34

SDS = Sodium dodecyl sulphate, HTC = hexadecyltrimethylammonium chloride, DMFU = 2,5-dimethylfuran.

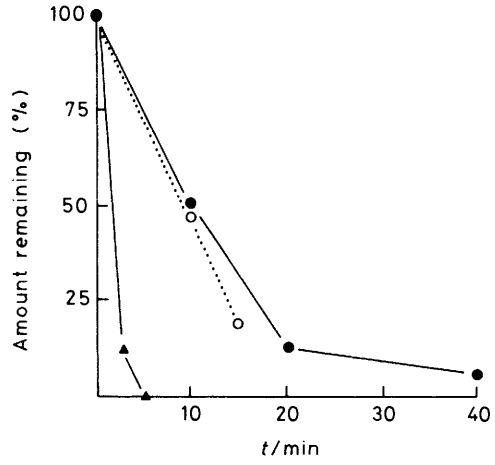


Figure 1. Photoreaction of chlorpromazine under various conditions: ○, CPZ–H_2O–O_2; ●, CPZ–Brij 58–O_2; ▲, CPZ–Brij 58–N_2

column (22 × 300 mm) packed with Merck 60 silica gel (70—230 mesh) and eluted with a solvent mixture of ethyl acetate–ethanol–water (8 : 3 : 3) using an FMI pump. The eluant was monitored using an ISCO UA-5 absorbance monitor. Freshly packed columns were found to be essential.

T.l.c. was done using the same eluant as above and Merck silica gel 60 F-254 plates.

The structure of the isolated promazine and its purity were established by comparison of the t.l.c., u.v., and ^1H and ^{13}C n.m.r. spectra with those of authentic promazine.

Results

Because quantification of the yields of the photoproducts is difficult, previous reports did not provide such data. We duplicated the conditions of these previous irradiations, both to provide such quantitative data and to confirm that any variation in the photochemistry of CPZ in micelles was due to the micelle and not another experimental variable. We confirmed that the photolysis of CPZ under nitrogen in water produces two major monomeric photoproducts, promazine (2) (38%) and 2-hydroxypromazine (3) (5%). Promazine was compared with authentic material and hydroxypromazine with the data of Huang and Sands.[3] The accurate quantification of the ' polar ' material, previously described as dimeric and polymeric,[3] was not feasible. Under all other conditions of irradiation the yield of 2-hydroxypromazine was only *ca.* 1%

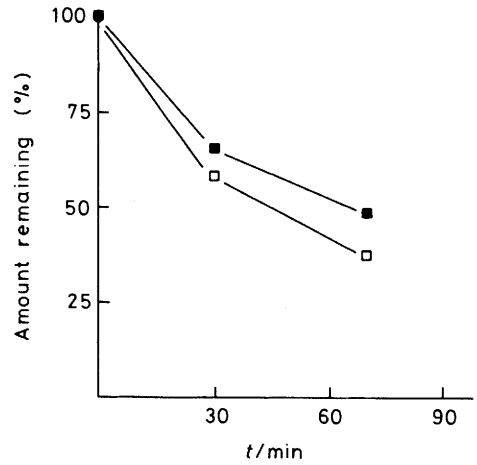


Figure 2. Photoreaction of promazine under various conditions: □, PRO–H_2O–O_2; ■, PRO–Brij 58–O_2

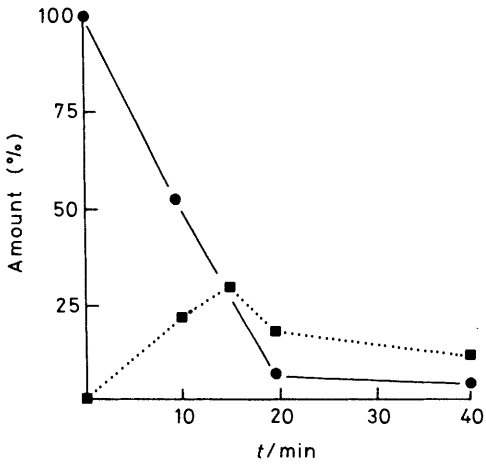


Figure 3. Formation of PRO from CPZ by photolysis in Brij 58 under O_2: ■, PRO formed; ●, CPZ remaining [2]

or less, so we focused on the quantification of promazine, which dramatically reflected the variety of medium effects noted in previous CPZ photochemistry, as well as the micelle effect. As in previous reports we found that when an air-saturated solution of CPZ in water was irradiated the number of products increased substantially, and no detectable (<2%) promazine was formed.

When a nitrogen-saturated aqueous solution of CPZ containing a detergent was irradiated the photolysis proceeds very much as in water without the surfactant (except for the diminished 2-hydroxypromazine yield); promazine was formed in 45% yield, and no new products were apparent.

The most dramatic observation involved irradiation of CPZ under oxygen in water containing a detergent. Again, promazine was found to be a major product (Table). In all previous irradiations of CPZ the presence of oxygen had caused photooxidation to predominate, but in the presence of detergents promazine was formed as if the oxygen were not present.

We also noted the effect of conditions on the rate of reaction of CPZ (Figure 1). The fastest reaction occurred when CPZ was irradiated under nitrogen in a solution containing detergent, but there was little difference between the rate of reaction under oxygen when Brij 58 was present or absent.

Irradiation under oxygen causes the promazine photo-

product to undergo secondary photoreaction. Figure 2 summarizes this observation, and points out why the yield of promazine is at a maximum before all of the CPZ starting material has completely reacted (Figure 3). It was observed that this photo-oxidation of promazine was also somewhat slower when detergents were present.

The last entries in the Table show that the presence of 2,5-dimethylfuran during irradiation did not significantly affect the yield of promazine isolated; neither did it appear to affect the rate of disappearance of CPZ.

To determine whether the detergents were affecting the singlet state reactivity of CPZ a variety of fluorescence spectra were taken. The intensity of emission of CPZ in water was virtually the same when detergent was present and the solutions were degassed with nitrogen. Oxygen decreased the intensity of fluorescence of the aqueous solution by 35%; with Brij 58 present the intensity diminished by 27%; with SDS present the intensity was diminished by 20% with oxygen.

Discussion

Many factors suggest that CPZ *in vivo* will predominantly exist either protein-bound, complexed, or in a liphophilic–hydrophobic environment. The parallel between micelles and such environments has frequently been noted.[11] Our results show that in such an environment the presence of oxygen does not stop the photodehalogenation of CPZ, even though it does so in aqueous solutions. The various types of biological damage caused by CPZ photolysis have been clearly summarized by Kochevar,[8b] and include cell lysis and covalent bond formation with DNA and proteins. That photo-oxidation of CPZ swamps all other photoreactions in water has led many to feel that the mechanism(s) for biological damage should incorporate this phenomenon. Our results suggest that this conclusion is premature. While photo-oxidation cannot be ruled out as a contributor to biological damage neither can oxygen-independent reactions.

The origin of the protection of the CPZ from photo-oxidation when in a micelle could be a combination of factors. Some contribution may be the protection from singlet state reactions–interactions with oxygen as evidenced by the fluorescence studies, though this is probably minor. Turro [12] has examined the dynamics of oxygen quenching of singlet excited states of hydrocarbons in micelles and found the quenching constants to be about two orders of magnitude lower than those of fluid solutions, because of the high internal microviscosity in micelles. Paralleling this diminution of the quenching rate constants of fluorescent probes in micelles are studies showing similarly diminished quenching rates in solutions containing proteins,[13] DNA,[14] and vesicles.[15] It would appear that the micelle model exactly parallels the behaviour of excited states in biological environments with respect to rate of quenching of fluorescence by molecular oxygen, and it seems reasonable that it may also mirror the manner in which complexation of CPZ *in vivo* may lead to a photoreaction that does not involve oxygen.

A recent observation by Moore [16] that CPZ irradiation in surfactant solutions leads to enhanced rates of oxygen uptake led to renewed interest in the role of photosensitized oxidation as the cause of the CPZ photosensitivity. However, without performing product analyses the authors were forced to conclude that ' it is not clear whether the free radical (Type I) or the $^1O_2$ (Type II) mechanism is the more important.' [16] Our study now shows that the predominant mode of photoreaction of CPZ in micelles with oxygen present continues to be dehalogenation. It is not at all clear whether the presence of oxygen leads to formation of singlet oxygen, electron transfer, or formation of an exciplex of oxygen and CPZ. Moore's results are consistent with chain processes leading to oxygen incorporation, or even reaction with oxygen of some of the radical intermediates formed from dehalogenation. Some of these oxygen-consuming reactions may lead to toxic products or be prone to induce biological damage, but it would be unwise to exclude from consideration the probable involvement of the radicals from dehalogenation.

Moore's [16] observation of an increased rate of chloride ion liberation on irradiation in cetamacrogol points out a second effect of the micelles. This increase in the quantum efficiency could either arise from the slowing of competitive processes to photoreaction, or by enhancement of the rate of the steps involving photoreaction. First, the higher internal microviscosity of micelles may slow the rate of bimolecular (quenching) processes of the reactive [15] triplet state (*e.g.* see ref. 17). This is apparently the manner in which micelles make it possible to observe readily the phosphorescence of many compounds in solution at room temperature.[18] Second, micelles might enhance the rate of photoionization of CPZ; the yield of the radical cation from irradiation of the parent chromophore, phenothiazine (PTH), has been found to be higher in micellar solutions.[19] It is firmly established that such

$$PTH \xrightarrow{h\nu} PTH^{+\cdot} + e^- \quad (1)$$

a radical cation is also formed from photodissociation of CPZ [5,20] and it is likely that the parallel behaviour of CPZ and PTH observed by Navaratnam [21] will be found to extend to the effect of micelles on their photoionization. However, it is difficult to rationalize how this fits with the convincing evidence of Bunce [22] that the radical anion of CPZ is the intermediate that undergoes dehalogenation. Indeed, Navaratnam [20] formed the same conclusion in a parallel study from entirely different evidence, where he concluded that one path of reaction of hydrated electrons with CPZ led to dehalogenation. Consequently, micelle-induced enhancement of the efficiency of dehalogenation of CPZ [abbreviated in reaction (2) as Cl-PRO], might arise from an enhancement of step (1). It is important to note that in aqueous solution oxygen does *not* play a significant role in photoionization,[20] though it may be a reactant with species such as the various radicals shown in (1) and (2) (*cf.* ref. 6).

$$e^- + \text{Cl-PRO} \longrightarrow \text{Cl-PRO}^{\cdot-} \longrightarrow Cl^- + \text{PRO}^{\cdot} \quad (2)$$

An alternative way in which photoreaction may become more efficient is by increased importance of an intramolecular charge-transfer exciplex involving the amino-side-chain and the aromatic nucleus.[22] This intramolecular process could become more important because of the diminution of the importance of the bimolecular processes.

Finally, we should point out that the increased yields of PRO in micelles might arise because abstraction of a neighbouring hydrogen is much faster than the diffusional reaction of PRO· (the precursor) with oxygen. This result seems a more reasonable parallel of the reactivity of PRO· formed in a protein or membrane (from complexed CPZ) than would the result from a fluid aqueous solution, where easily abstractable hydrogen atoms or reactive π systems (which might be alkylated by radicals) would not be encountered.

Acknowledgements

We are grateful to the National Institutes of Health (A112200) for financial support.

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Received 15*th October* 1982; *Paper* 2/1771