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Spin trapping studies of photochemical reactions

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Abstract - Spin trapping is a very useful technique to study the photochemistry of chemical photosensitizers that undergo electron or hydrogen-transfer (Type I) reactions upon UV or visible irradiation. The Type I photochemistry of chlorpromazine [2-chloro-N-(3-dimethylamino-propyl)-phenothiazine], 3,3'4'5-tetrachlorosalicylanilide and 3,4',5-tribromosalicylanilide has been investigated using 2-methyl-2-nitroso-propane (MNP) and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) as spin traps. All compounds underwent dehalogenation upon UV-irradiation and the resultant aryl radicals were trapped and identified.

INTRODUCTION

Light is known to interact with chemical agents present in exposed tissues, such as the skin or eyes, to produce photosensitization (1). The chemical agent may be endogenous (protoporphyrin in erythropoietic protoporphyria), a drug (sulfonamides, declomycin, chlorpromazine), a topical agent (4-aminobenzoic acid and its esters in sunscreens; halogenated salicylanilides in soaps) or an environmental agent (polycyclic aromatic hydrocarbons in coal tar; amyl esters of 2-aminobenzoic acid in printer's ink) (2,3). The photosensitivity response may be one of two types, phototoxic or photo-allergic. The phototoxic reaction generally occurs during a subject's first exposure to sunlight, after the administration or topical application of a chemical or drug, and usually takes the form of an exaggerated erythemal response ("sunburn"). Photoallergic individuals may also exhibit an initial erythemal reaction. As this subsides, delayed abnormal responses may begin to appear including papular, eczematous and urticarial reactions. Such reactions to light may persist for months after avoidance of the photoallergen.

The initial step in all forms of photosensitivity must be the absorption of light by the chemical or its metabolites. When the ground state (S_0) of a molecule absorbs a photon of light it is converted to a very short-lived singlet excited state ($^1S^*$) which can rapidly convert to a long-lived excited triplet state ($^3S^*$). Most photosensitized reactions in biological systems are mediated by the triplet state of the sensitizer. Subsequent reactions can proceed by a number of different pathways, depending on the chemical nature of the sensitizer and the substrate as well as the reaction conditions. Two major types of processes may occur, termed Type I and Type II. In Type I (free radical or redox) reactions the triplet sensitizer molecule may abstract an electron (or hydrogen atom) from the substrate molecule (R) to give a semi-reduced (free radical) form of the ($S^{\bullet -}$) and a semi-oxidized (free radical) form of the substrate ($R^{\bullet +}$). These processes may be summarized as follows:

$$S_0 \xrightarrow{h\nu} {}^1S^*$$

$$^1S^* \longrightarrow {}^3S^*$$

$$^3S^* + R \longrightarrow S^{\bullet -} + R^{\bullet +}$$

The semi-reduced form of the sensitizer may also react directly with oxygen to give the superoxide radical ($O_2^{\bullet -}$)

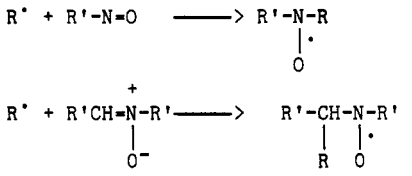
$$S^{\bullet -} + O_2 \longrightarrow S + O_2^{\bullet -}$$

In Type II (energy transfer) reactions, electronic excitation energy is transferred from the triplet state sensitizer to ground state oxygen to give a highly electrophilic excited singlet state ($^1O_2$) of oxygen:

$$^3S^* + {}^3O_2 \longrightarrow S_0 + {}^1O_2$$

This form of oxygen reacts with many kinds of biomolecules much more rapidly than ground state oxygen.

Free radicals from Type I reactions may be detected, identified and quantitated by electron spin resonance (ESR) spectroscopy. However, since most free radicals generated photolytically are chemically reactive, they cannot always be observed by direct ESR. For such radicals the technique of spin trapping may be employed (4). Spin trapping is a technique that involves the addition of a reactive free radical (R$^{\bullet}$) to an organic diamagnetic nitrone or nitroso compound (spin trap) to form a more stable nitroxide free radical (spin adduct):



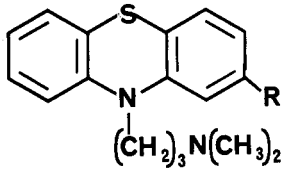
The structure of the parent free radical (R$^{\bullet}$) may then be determined from the hyperfine couplings of the ESR spectrum of the resultant spin adduct. Nitrones have the advantage that they are highly reactive and give rise to stable adducts. Their main disadvantage is that they provide less information. On the other hand nitroso spin traps generally produce adducts that have unique spectra. Unfortunately nitroso compounds are often photochemically active and their adducts are less stable.

Singlet oxygen from Type II reactions may also be detected by ESR. One technique employs a hindered amine (2,2,6,6-tetramethylpiperidine) which upon reaction with singlet oxygen yields a stable nitroxide free radical (2,2,6,6-tetramethylpiperidine-1-oxyl, TEMPO). A second procedure involves the measurement of oxygen consumption by monitoring the effect on the linewidth of an exogenously added nitroxide eg. TEMPO. In both cases the presence of singlet oxygen is confirmed by the use of "specific" quenchers such as dibenzofuran, dimethylfuran or DABCO. Singlet oxygen may also be detected directly by observing its luminescence at 1270 nm.

SPIN TRAPPING STUDIES

Chlorpromazine

Chlorpromazine [2-chloro-N-(3-dimethylaminopropyl)phenothiazine] (1) is a frequently prescribed antipsychotic drug which causes both phototoxic and photoallergic reactions (5,6). Irradiation of chlorpromazine in solution is known to produce the chlorpromazine cation radical, as well as other photoproducts which may go through radical intermediates (6).



1 R = Cl

2 R = H

3 R = •

UV irradiation of 1 in aqueous solution under anerobic conditions resulted in the generation of an aryl radical (3) which was readily trapped by 2-methyl-2-nitrosopropane (MNP) (7). No radicals were trapped during the irradiation of promazine (2). Our results suggest that radical 3 is sufficiently reactive to account for the observation that chlorpromazine is more phototoxic than its parent promazine. In the presence of oxygen both chlorpromazine and promazine form a superoxide-dismutase-insensitive oxygen-centered intermediate which, when trapped by 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), rapidly decays to DMPO-OOH and subsequently to DMPO-OH. Chlorpromazine readily undergoes photoelectron ejection only when it is excited into the second excited singlet state (λ <280 nm). This previously unknown wavelength dependence of photoionization does not occur in the parent promazine. We have shown by laser flash photolysis that the often-observed photoionization which takes place when chlorpromazine is excited into the lowest excited singlet state (λ >310 nm), is a biphotonic process involving the triplet state, contrary to previous reports (8). This suggests that formation of the cation radical via photoionization is not important in chlorpromazine photosensitization by sunlight.

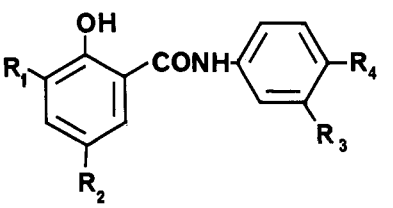
A spin-trapping investigation of chlorpromazine sulfoxide, a metabolite formed in man and several other mammalian species, showed that when irradiated with near UV light, the sulfoxide produces large amounts of the highly reactive hydroxyl radical ($^{\bullet}$OH) as well as the cation radical (9). To further examine the role of the sulfoxides in phenothiazine phototoxicity, measurements of their fluorescence and phosphorescence spectra and lifetimes

were carried out. Although the UV absorption spectra of chlorpromazine and its sulfoxide overlap, their fluorescence spectra are distinctive and well separated. By monitoring the fluorescence, we were able to follow the conversion of chlorpromazine to its sulfoxide in air-saturated aqueous solution, as well as the photolysis of sulfoxide back to chlorpromazine under anerobic conditions in the presence of ascorbate (vitamin C). Animal species which do not form the sulfoxide metabolically do not develop ocular complications, and thus it seems likely that the $^{\bullet}OH$ radical generated from the sulfoxide may be responsible for ocular toxicity.

The role played by singlet oxygen in phenothiazine-induced photosensitivity remains controversial (6). Direct measurements of luminescence at 1270 nm in our laboratory have shown that singlet oxygen production by photoexcited chlorpromazine and promazine is greatest in hydrophobic solvents (benzene, hexane, cyclohexane) and weakest in hydroxylic organic solvents (ethanol, methanol). There is no detectable singlet oxygen emission from photo-irradiated aqueous solutions of chlorpromazine or promazine. Thus, Type II photosensitization in vivo may occur only when these drugs are present in nonaqueous locations such as cell membranes.

Halogenated salicylanilides

Several antibacterial halogenated salicylanilides, including 3,3',4',5-tetra-chlorosalicylanilide (TCSA) (4) and 3,4',5-tribromosalicylanilide (TBSA) (5) are known to cause photoallergy in human and animal subjects (3). Since the salicylanilides are practically



4 $R_1=R_2=R_3=R_4=Cl$

5 $R_1=R_2=R_4=Br$, $R_3=H$

6 $R_1= -N(O)C(CH_3)_3$, $R_2=R_4=Cl$, $R_3=H$

insoluble in aqueous solutions at neutral pH, all aqueous spin trapping studies were carried out in 0.1 N NaOH. When TCSA was irradiated (λ = 356 nm) in the presence of MNP an adduct exhibiting a broad triplet was detected by ESR (Table 1). This adduct (6) was probably formed by the reaction of MNP with the aryl radical generated by the loss of the 3-chloroatom from TCSA. The absence of additional hyperfine couplings from the aromatic ring of 1 is due to steric interaction between the nitroxide moiety and the adjacent hydroxyl group which rotates the nitroxide out of the plane of the aromatic ring (10).

TABLE 1. ESR parameters of adducts formed during the irradiation (λ > 300 nm) of the salicylanilides

Salicylanilide	Spin Trap	Hyperfine Splitting (G) a_N	a_H	a_X
TCSA	MNP	15.6	-	-
TBSA[a]	MNP	15.5	-	-
TBSA[b]	MNP	14.4	2.0(2)	0.9(2H)
4'-Bromosalicylanilide	MNP	14.4	2.0(2)	0.9(2H)
TBSA + N-acetylcysteine	DMPO	15.0	16.8	-
TBSA + cysteine	DMPO	15.2	17.0	-
TBSA + glutathione	DMPO	15.2	16.2	-
TCSA + glycylglycine	MNP	15.9	2.3	2.5(1N)

[a]ESR spectrum recorded immediately upon irradiation
[b]ESR spectrum recorded after 16 min irradiation

Irradiation (λ = 356 nm) of TBSA with MNP initially gave a broad triplet similar to that observed for TCSA (Table 1; Fig. 1A). However, upon further irradiation a 21-line ESR spectrum was observed (Fig. 1B). Since a very similar spectrum was obtained from 4'-bromosalicylanilide (Fig 1C), the spin adduct derived from TBSA must arise from loss of a bromine atom from the 4'-position.

The photoallergic response to the salicylanilides is thought to be due to the photochemical generation of chemically reactive species that covalently bind to proteins or other macromolecules to form antigens. Since aryl radicals are highly reactive their reaction with amino acids and peptides was examined. De Vries and van Henegouwen (11) have reported that, when TBSA is irradiated in the presence of glutathione, a photoconjugate, 3-glutathiyl-4',5-dibromosalicylanilide is formed. These workers proposed that the glutathione thiyl radical was a key intermediate in this photoreaction. The ability of TBSA to generate sulfur-centered radicals from sulfhydryl containing compounds was teste using DMPO as a spin trap (Table 1). Irradiation of TBSA with DMPO and N-acetylcysteine resulted in the trapping of the N-acetylcysteine thiyl radical (Table 2). No radicals were trapped when TBSA or N-acetylcysteine were irradiated alone or in the presence of DMPO or when

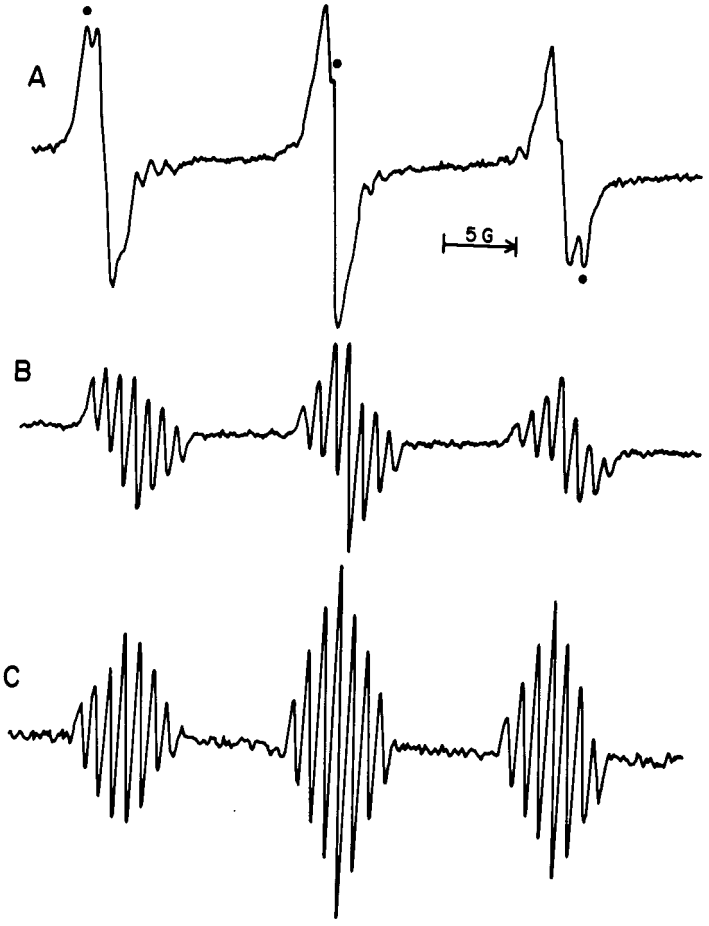


Figure 1. The ESR spectra of bromosalicylanilides irradiated (λ = 356 nm) in 0.1N NaOH in the presence of NMP. A) TBSA, initial spectrum. B) TBSA, final spectrum. C) 4'-bromosalicylanilide. • = di-tert-butylnitroxide.

TABLE 2. Free radicals formed during irradiation (λ > 300 nm) of TCSA with amino acids and peptides.

Amino acid or peptide	Predominant radical trapped by MNP
Gly-Ala	$H_2\dot{N}CHCONHCH(CH_3)COOH$
Gly-Val	$H_2NCH_2CONHCH(COOH)\text{-}\dot{C}(CH_3)_2$
Gly-Gly	$H_2NCH_2CONH\dot{C}HCOOH$
Gly-Leu	$H_2NCH_2CONHCH(COOH)CH_2\text{-}\dot{C}(CH_3)_2$
Gly-Ser	$H_2NCH_2CONHCH(COOH)\text{-}\dot{C}HOH$

salisalicylanilide was used instead of TBSA. Irradiation of TBSA and DMPO with cysteine or gluglutathione gave the corresponding thiyl adducts of the spin trap (Table 1). Similar results obtained with TCSA.

In addition to reacting with sulfhydryl compounds, the aryl radical from TCSA can abstract hydrogen atoms from peptides (Tables 1 and 2). This reaction was found to be highly specific in that it was observed for peptides derived from alanine, glycine, valine, leucine and serine but not with those containing histidine, threonine and aspartate (Table 2). For dipeptides containing simple amino acids, e.g. alanine and glycine, the predominant reaction was abstraction of a hydrogen atom from the α-carbon atom, while for others, e.g. leucine and serine, hydrogen abstraction also occurred in the sidechain (Table 2). No reaction occurred with the free amino acids.

DISCUSSION

We have shown that chlorpromazine (ArCl), TCSA (ArCl) and TBSA (ArBr) undergo facile photodehalogenation, via a mechanism that involves homolytic bond fission, with the concomitant generation of highly reactive aryl radicals (Ar$^{\bullet}$). The latter can abstract hydrogen atoms from sulfhydryl-containing compounds (RSH) or peptides (PH)

$$\mathrm{ArBr(Cl)} \xrightarrow{h\nu} \mathrm{Ar^{\bullet} + Br^{\bullet}(Cl^{\bullet})}$$
$$\mathrm{Ar^{\bullet} + RSH} \longrightarrow \mathrm{RS^{\bullet} + ArH}$$
$$\mathrm{Ar^{\bullet} + PH} \longrightarrow \mathrm{P^{\bullet} + ArH}$$
$$\mathrm{Ar^{\bullet} + RS^{\bullet}} \longrightarrow \mathrm{RSAr}$$
$$\mathrm{Ar^{\bullet} + P^{\bullet}} \longrightarrow \mathrm{PAr}$$

or peptides (PH) to form secondary radicals. While all these studies were carried out *in vitro* it appears possible that similar reactions may occur *in vivo*. The reaction of secondary protein radicals with the primary aryl radicals would result in covalent binding of these drugs to give molecules with antigenic potential. The difference in photosensitizing potential between chlorpromazine and promazine may be due to the ability of the former to generate reactive aryl radicals upon UV-irradiation.

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