Photochemical Generation of Superoxide Radical Anion in Water

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Superoxide radical anion ($O_2^-\cdot$) formed photochemically in aqueous solutions of aromatic amines (aniline, anthranilic acid, and N-acetylanthranilic acid), amino acids (tryptophan, kynurenine, and tyrosine), p-cresol, and acetophenone. $O_2^-\cdot$ was detected with the enzyme probe superoxide dismutase. Chemicals that generated $O_2^-\cdot$ also sensitized the photodechlorination of chloroacetic acid; indole and indole derivatives (tryptophan and tryptophol), p-cresol, tyrosine, anisole, and aniline were active as sensitizers in 300-nm light. Photodechlorination sensitized by tryptophan was more efficient in deoxygenated solutions, consistent with the view that the displacement of chloride was due, in part, to hydrated electrons. Photochemical mechanisms for $O_2^-\cdot$ production in near-UV light are believed to involve photoionization of the sensitizer or complete electron transfer in charge-transfer quenching of excited states of the sensitizer or molecular oxygen.

Our previous studies on the sensitized photooxidation of pesticides in water demonstrated the key role played by photochemically generated hydrogen peroxide (Draper and Crosby, 1981). Tryptophan and methylene blue were the subject of this initial investigation, although we later found that stable photochemical oxidants were formed by other aromatic compounds in water as well. Hydrogen peroxide also was formed photochemically in natural waters (Draper and Crosby, 1983).

We describe here further studies on the photochemical production of hydrogen peroxide by organic compounds in water. In studying this phenomenon our objectives were to determine the involvement of oxygen, the solvated electron (e_{aq}^-), and superoxide radical anion ($O_2^-\cdot$).

MATERIALS AND METHODS

Chemicals. N-Acetylanthranilic acid was prepared by treatment of anthranilic acid with acetic anhydride in glacial acetic acid; a flocculent precipitate was collected, washed with water, and crystallized from ethanol–water (mp 186–187 °C). Chloroacetic acid was recrystallized from carbon tetrachloride and dried in a vacuum desiccator over phosphorus pentoxide (mp 59–60 °C). Recrystallization of the commercial material reduced its free chloride content from 0.05 to 0.015 mol %. Other chemicals were reagent grade obtained through commercial sources. Superoxide dismutase enzyme (SOD, 2900 units/mg of protein) was obtained from Sigma Chemical Co. Aqueous solutions were prepared in distilled water which was purified by passage through XAD-4 macroreticular resin (Rohm and Haas Co.).

Irradiation. In studies with SOD, solutions were irradiated in a large-scale photoreactor (Crosby and Wong, 1973) fitted with six F40BL fluorescent lamps (Sylvania) which exhibit maximum energy output between 290 and 450 nm; with borosilicate reaction vessels the near-UV cutoff for the system was ~285 nm. The light flux in the center of the chamber was 600 $\mu W/cm^2$ and solution temperatures did not exceed 30 °C. For photodecomposition studies of chloroacetic acid, a Rayonet photoreactor (Southern New England Ultraviolet Co.) fitted with RPR-3000 (maximal energy output at 300 nm) or RPR-3500 (maximal output at 360 nm) lamps was used; samples

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were sealed in borosilicate tubes and held in a merry-go-round apparatus.

SOD and Photooxidant Yield. Organic compounds (10 mM) were irradiated in 1 mM, pH 7.0, phosphate buffer for 6 h in sealed borosilicate flasks with or without 0.04 mg/mL (116 units/mL) SOD. Controls were held in the dark to monitor autoxidation reactions. Oxidant levels were determined without delay by the sensitive spectrophotometric assay of Mottola et al. (1970). The limit of detection for hydrogen peroxide was approximately 2 μM.

Photodechlorination of Chloroacetic Acid. Concentrated aqueous solutions of chloroacetic acid, 23.1 g/L (245 mM), were irradiated with 0.5 g/L of the following: aniline (5.4 mM), anisole (4.6 mM), p-cresol (4.6 mM), indole (4.3 mM), L-tryptophan (2.45 mM), tryptophol (3.8 mM), and L-tyrosine (2.8 mM). Dark control solutions were maintained to monitor hydrolysis, and direct photodechlorination was determined by irradiating chloroacetic acid in demineralized water.

Chloride concentrations were determined electrochemically by using a solid-state ion-specific electrode (Orion Research, Inc.). Aqueous samples (50 mL) were combined with 1 mL of 5.0 M sodium nitrate and the electrode was allowed to equilibrate in the stirring sample for 60 s. Chloride determinations were done in triplicate and the electrode was calibrated relative to electrode potentials in aqueous sodium chloride ranging from 10^{-5} to 10^{-2} M.

Oxygen concentrations were controlled in some experiments. Aqueous solutions were deoxygenated by triplicate freeze–thaw cycles under nitrogen; after each freezing at −80 °C the vessel headspace was sparged with purified nitrogen. Solutions were oxygenated by passing purified oxygen through an immersed fritted tube for 10 min with a flow rate of approximately 1.5 L/min.

RESULTS AND DISCUSSION

Oxygen Requirements. Tryptophan is stable in near-UV light under anaerobic conditions or in oxygenated solutions held in the dark. The absorbance spectrum of tryptophan was unchanged and no hydrogen peroxide formed under these conditions. In oxygen-saturated solutions, however, tryptophan photodecomposed concomitant with the formation of hydrogen peroxide (301 μM after 21 h of treatment); the photolysis mixtures were yellow and showed diminished absorption at 281 nm (λ_{max} for tryptophan) and increases in absorptivity at 240 nm and above 300 nm. Approximately 20% of the dissolved oxygen underwent reduction to the level of hydrogen peroxide; this estimate assumes initial oxygen saturation.

Table I. Superoxide Dismutase Effects on Photochemical Oxidant Yield

compound	absorption maxima[a]		oxidant concn, μM[b]		
	nm	e	dark	light	light, SOD
acetophenone	278 320 A[c]	1000 50	nd[d]	4.0	13
N-acetyl- anthranilic acid			nd	nd	7.5
aniline	280 W[e]	1600	nd	7.5	39
anthranilic acid	337 A	5000	nd	6.5	22
p-cresol	280 A	1600	nd	4.5	120
hydroquinone	294 A	3200	3.0	3.5	3.5
kynurenine			nd	6.0	150
tryptophan	281 A	6300	nd	180	270
tyrosine	275 W	1000	nd	9.0	12

[a] Hirayama (1967). [b] Concentration of hydrogen peroxide yielding an equivalent response. [c] Alcohol. [d] Not detected. [e] Water.

$O_2^-\cdot$ Production by Model Compounds. SOD reacts rapidly with $O_2^-\cdot$ catalyzing its dismutation [$k = 2 \times 10^9$ M^{-1} s^{-1} (Fridovich, 1972)]. The spontaneous disproportionation of $O_2^-\cdot$ at pH 7.0 is considerably slower [$k = 6 \times 10^5$ M^{-1} s^{-1} (Behar et al., 1970)]. SOD sharply increased the yield of stable photooxidant formed by most aromatic compounds in water (Table I). The increases observed varied greatly for each compound. For tryptophan, for example, enzymic catalysis increased hydrogen peroxide production by a factor of only 1.5, but a 25-fold increase was observed when p-cresol or kynurenine was irradiated with SOD. SOD was used previously to demonstrate the photochemical generation of $O_2^-\cdot$ in solutions of tryptophan (McCormick and Thomason, 1978).

Quantum yields for $O_2^-\cdot$ have not been determined in this study and detailed comparisons of the activities of these compounds are not possible. As with any photochemical process, the spectral overlap of the light source with the chromophore determines the reaction rate; absorption maxima and molar absorptivities for each chromophore appear in Table I. It is clear from these data, however, that p-cresol is an extremely efficient $O_2^-\cdot$ generator; its molar extinction coefficient is only one-fourth that of tryptophan yet its yield of $O_2^-\cdot$ approached half that of the amino acid. It also appears that electron-withdrawing groups diminish activity since aniline was more active than anthranilic acid or N-acetylanthranilic acid.

Photodechlorination of Chloroacetic Acid. The photoionization of aromatic compounds in UV light is well documented (Grossweiner and Joschek, 1965). Hydrated electrons have been detected, for example, in flash photolysis studies of p-cresol, aniline, and tryptophan. Dissolved oxygen scavenges e_{aq}^- in an extremely fast reaction, yielding $O_2^-\cdot$ [$k = 2 \times 10^{10}$ M^{-1} s^{-1} (Baxendale, 1967)]. Solvated electrons also react with alkyl chlorides, causing dissociation to chloride ions and alkyl radicals, and for chloroacetic acid this reaction is rapid ($k = 1.2 \times 10^9$ M^{-1} s^{-1}).

To test for hydrated electrons, we irradiated model compounds known to generate e_{aq}^- in UV light with chloroacetic acid and monitored the production of chloride. Precise chloride determination was possible at concentrations as low as 10 μM as indicated by electrode potentials for aqueous sodium chloride (Figure 1). At higher chloride concentrations (100 μM–10 mM), the electrode potential was consistent with the Nernst equation (~57 mV/decade). A high concentration of chloroacetic acid was present initially in these experiments to minimize the competing reactions of e_{aq}^-.
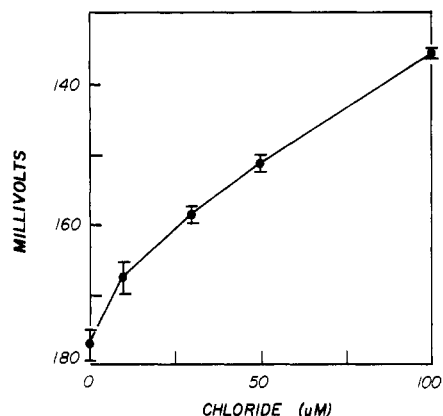

Figure 1. Standard response curve for the chloride-specific electrode in aqueous sodium chloride solutions. Error bars indicate ± one standard deviation.
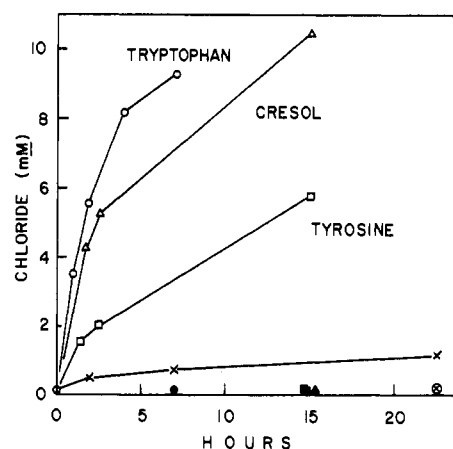

Figure 2. Photodechlorination of chloroacetic acid in tryptophan (O), p-cresol (△), and tyrosine (□) solutions and demineralized water (X). Filled symbols (●, ▲, ■, ⊗) represent dark controls. Air-saturated solutions were irradiated in 300-nm light.

Table II. Sensitized Photodechlorination of Chloroacetic Acid

sensitizer	Δ[chloride], mM	
	300-nm lamps[a]	360-nm lamps[b]
none	0.76	0.62
aniline	2.4	0.64
anisole	5.0	0.62
p-cresol	11.5	0.91
indole	10.5	1.1
tryptophan	11.0	1.2
tryptophol	12.5	3.7
tyrosine	4.5	0.88

[a] After 11 h of treatment; chloride concentrations were 0.04 mM initially and increased to 0.05 mM in each dark control due to hydrolysis. [b] After 14 h of treatment; chloride concentrations were 0.08 mM initially and increased to 0.09 mM in control solutions held in the dark.

Photodechlorination of chloroacetic acid was slow in near-UV light (Figure 2) and after 11 h of irradiation (300 nm, air-saturated solutions) less than 0.4% of the chloroacetic acid was converted to free chloride ion. The release of chloride was rapid initially, but the rate declined with continued irradiation. In one experiment the chloride concentration increased from 0.105 to 0.400 mM in the first hour of irradiation (300 nm), but after 7 h the rate was decreased to 10% of the initial value. The rapid photochemical production of chloride initially may be due to the photodecomposition of chlorinated impurities; purification of the monochloroacetic acid sample studied was limited

Table III. Effect of Oxygen on the Direct and Sensitized Photodechlorination of Chloroacetic Acid[a]

sensitizer	oxygen	length of treatment, h	Δ[chloride], mM[b]
none[c]	−	8.5	0.09
none[c]	+	8.5	1.57
tryptophan[d]	−	0.25	0.70
tryptophan[d]	+	0.25	0.40
tryptophan[d]	−	3	3.2
tryptophan[d]	+	3	1.6

[a] 300 nm. [b] Corrected for hydrolysis measured in dark controls. [c] [Chloride] was 0.055 mM initially and increased to 0.078 mM due to hydrolysis. [d] The chloroacetic acid solution was preirradiated for 22 h before addition of tryptophan; [chloride] was 1.25 mM upon addition of the sensitizer and hydrolysis was undetectable in the dark control.

to recrystallization. The direct photolysis rate was not greatly effected by wavelength with photodechlorination occurring in 360- or 300-nm light (Table II); chloroacetic acid absorbs considerably more strongly in UV light (ϵ_{254} = 1.4 L M^{-1} cm^{-1}, water) than in near-UV light (300 or 360 nm) where extinction coefficients are less than 0.2 L M^{-1} cm^{-1}. Previous studies of chloroacetic acid in UV light (185–254 nm) have demonstrated photodechlorination by hydrolysis of an excited state or homolytic cleavage of the C–Cl bond (Neumann-Spallart and Getoff, 1979).

Direct photodechlorination in chloroacetic acid solutions was stimulated by oxygen by an undefined mechanism. Under a nitrogen atmosphere the photolysis rate only slightly exceeded that of hydrolysis with a 95% reduction in photodechlorination (Table III).

Compounds which generated superoxide radical anion in near-UV light also sensitized the photodechlorination of chloroacetic acid (Figure 2). Indoles and p-cresol were efficient sensitizers, causing a 12–16-fold increase in the yield of free chloride in 300-nm light after 11 h of treatment. Aniline, anisole, and tyrosine also were active in 300-nm light, but in 360-nm light only tryptophol sensitized the reaction (Table II). Sensitizers that most efficiently generated superoxide, i.e., p-cresol and tryptophan (Table I, light/SOD treatment), also were among the most active sensitizers for photodechlorination.

The yield of chloride ion exceeded the initial sensitizer concentration in anisole, p-cresol, indole, tryptophan, tryptophol, and tyrosine solutions (Table II); for example, over four chlorines were displaced for each tryptophan molecule undergoing photodecomposition. These data suggest that free radical chain reactions are responsible, in part, for the dechlorination of chloroacetic acid and that the dechlorination radical from chloroacetic acid or other radicals attacks the substrate. Sensitized photochemical dechlorination of chloroacetic acid, unlike direct photodechlorination, did not require oxygen (Table III). Hydrated electrons react preferentially with oxygen relative to chloroacetic acid ($k_{O_2}/k_{chloroacetic\ acid}$ = 17), but in these experiments chloroacetic acid was present in great excess. In air-saturated solutions the chloroacetic acid: oxygen ratio equaled almost 1000. Under these conditions the reaction of e_{aq}^- with chloroacetic acid would be diminished by approximately 2% on increasing oxygen from zero to its concentration in air-saturated solutions [$k_{O_2}[O_2]/$ ($k_{chloroacetic\ acid}$[chloroacetic acid]) = 0.018]. Oxygen, however, inhibits the photodechlorination reaction in tryptophan solutions by 43–50% (Table III).

We conclude that the dechlorination of the substrate is due to the reaction of solvated electrons formed on photoionization of the sensitizer and to subsequent free radical

$$D \xrightarrow{h\nu} D^{+\cdot} + e_{aq}^-$$

$$e_{aq}^- + O_2 \longrightarrow O_2^-$$
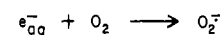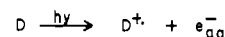
Figure 3. Photochemical generation of $O_2^-\cdot$ by photoionization of the sensitizer molecule D (mechanism 1).

$$^3D_I + RNH_2 \rightarrow {}^3D^{-\cdot}---RNH_2^{+\cdot} \rightarrow D^- + RNH_2^{+\cdot}$$

$$D^- + O_2 \longrightarrow O_2^- + D$$
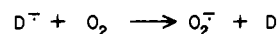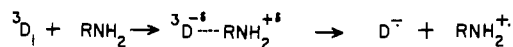
Figure 4. Photochemical generation of $O_2^-\cdot$ by a reducible sensitizer. D signifies the sensitizer molecule and RNH$_2$ represents an amine or other electron-rich substrate (mechanism 2).
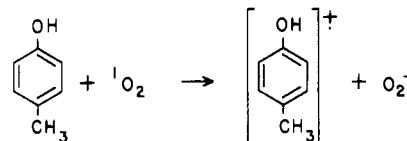
Figure 5. Proposed mechanism for the production of $O_2^-\cdot$ by electron-transfer quenching of singlet molecular oxygen (mechanism 3).

reactions. There are conflicting reports regarding the photoionization threshold of tryptophan; Amouyal et al. (1979), for example, found the photoionization threshold to be 275 nm in water. Our data, however, are in agreement with more recent reports (Mossoba et al., 1982) that tryptophan and tyrosine photoionize at 313 nm but not at 334 nm. Alternative mechanisms include dechlorination of the substrate by hydrogen atoms (Mossoba et al., 1982), which can be formed photochemically by tryptophan above 275 nm (Pailthorpe and Nicholls, 1971), or by direct electron transfer from the triplet state of tryptophan to the alkyl halide substrate.

Superoxide radical anion is an exceptionally strong nucleophile in aprotic solvents where its chemical reactions have primarily been examined (Roberts and Sawyer, 1981). Organic halides (i.e., benzyl bromide, allyl iodide, and allyl bromide) are known to undergo nucleophilic displacement reactions with electrochemically generated $O_2^-\cdot$ in propanol–water mixtures (Divisek and Kastening, 1975). In oxygenated solutions $O_2^-\cdot$ may contribute to photodechlorination of chloroacetic acid by nucleophilic displacement. The net effect of oxygen, however, is to inhibit dechlorination, possibly due to pretermination reactions which limit free radical processes.

Photochemical Mechanisms for $O_2^-\cdot$ Production. Our data are consistent with numerous previous studies demonstrating photoionization of tryptophan and other amino acids. We present evidence that other aromatic compounds (amines, phenols, methoxybenzenes, and indoles) photoionize in near-UV light as well. The formation of $O_2^-\cdot$ and its dismutation product, hydrogen peroxide, in solutions of these sensitizers is rationalized by the scavenging of hydrated electrons by dissolved oxygen (mechanism 1, Figure 3). Alternative mechanisms for $O_2^-\cdot$ production which do not involve hydrated electrons may be important for other classes of sensitizers.

Charge-transfer complexes between an excited acceptor and a ground-state electron donor molecule may decay by partial or complete electron transfer in polar solvents. An example is the charge-transfer quenching of triplet dyes by amines. Univalent oxygen reduction may occur by two distinct photochemical mechanisms of this sort: (mechanism 2) a photochemically excited sensitizer may undergo reduction to a radical anion, with $O_2^-\cdot$ formed by aut-

oxidation of this sensitizer radical (Figure 4); (mechanism 3) photochemically generated singlet oxygen may be quenched by complete electron transfer, yielding $O_2^-\cdot$ (Figure 5).

Mechanism 2 is supported by an extensive volume of literature. Flavin and its analogues, for example, undergo photochemical reduction to a semiquinoid form in the presence of an electron donor (EDTA), and spontaneous oxidation of the reduced dye with ground-state oxygen yields $O_2^-\cdot$ (Massey et al., 1969). The triplet state of methylene blue forms charge-transfer complexes with alkylamines resulting in electron transfer or spin inversion (Kayser and Young, 1976). Other electron carrying dyes including xanthenes, acridines, phenoxazines, and isoalloxazines are reduced photochemically as well, and in oxygenated solutions each may generate $O_2^-\cdot$.

Mechanism 3 is less substantiated. Charge-transfer mechanisms are proposed for the quenching of singlet oxygen by sulfides, amines, alkyl amines, methoxybenzenes, and phenols (Kacher and Foote, 1979 and references cited therein). These authors have concluded based on thermodynamic considerations that in each instance only partial electron transfer is involved in singlet oxygen quenching. Recently, however, Saito et al. (1981) demonstrated that $O_2^-\cdot$ was formed on quenching singlet oxygen with N,N-dimethyl-p-anisidine.

Electron-rich compounds including aromic amines, p-cresol, and methoxybenzenes were found to generate $O_2^-\cdot$ in this study. For these compounds, which are not likely to undergo photoreduction, mechanism 1 or 3 may be responsible for activity. In highly polar solvents electron carrying dyes, i.e., methylene blue (Draper and Crosby, 1981), can be expected to generate $O_2^-\cdot$ and its stable product, hydrogen peroxide, according to mechanism 2.

CONCLUSION

We have considered the photochemical production of superoxide anion by organic compounds in aqueous solution. Several classes of aromatic compounds were active, and this activity was readily detected with superoxide dismutase (and a suitable oxidant assay). Compounds which generated $O_2^-\cdot$ also sensitized the photodechlorination of chloroacetic acid, most likely due to the reaction of solvated electrons. Photoionization, therefore, seems to be an important process leading to $O_2^-\cdot$ although charge-transfer quenching mechanisms were proposed as alternatives. The ultimate yield of hydrogen peroxide from photochemically generated $O_2^-\cdot$ is determined by the rate of dismutation relative to competing reactions including reverse electron transfer. Photochemically generated superoxide may itself be an important reactant in photobiology and aquatic photochemistry.

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