Studies on Production and Chemical Property of Singlet Oxygen and Superoxide Radical by Dyestuffs

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Received 15 May 2009; Accepted 10 July 2009

Abstract: There are several low lying singlet oxygen ($^1\text{O}_2$) and superoxide radical (O_2^-) which are important in photochemical oxidation. In our present analysis we are studying chemical property of singlet oxygen ($^1\text{O}_2$) and superoxide radical (O_2^-) and some dyestuffs species to produce reactive oxygen such as singlet oxygen $^1\text{O}_2$ and superoxide radicals. Irradiation with sun light *in vitro* the dyestuff like benzanthrone, metanil yellow and *p*-aminodiphenylamine were found to produce reactive oxygen species such as singlet oxygen ($^1\text{O}_2$) and/or superoxide radicals (O_2^-). The dose response relationship between singlet $^1\text{O}_2$ production when sunlight expose of those dyestuffs (0-25 min), and (0-12 min) for super oxide (O_2^-) production were studied. However benzanthrone produces detectable amount of $^1\text{O}_2$. Although metanil yellow and *p*-aminodiphenylamine (*p*-ADPA) did not produce detectable amounts of $^1\text{O}_2$ under similar conditions. The above dye stuffs are routinely used in textiles, cosmetics, detergents, leather industries as well as food additives and role of these activated oxygen species in the development of skin diseases.

Keywords: Dyes, Benzanthrone, Photosensitization, Singlet oxygen, Superoxide radicals and Chemical property,

Introduction

Production of synthetic dyes in India about 25000 metric tons every year¹. Which are Industrial health hazards to people². Dyestuff industries have occupied a prominent place in the industrial organization of the modern world. One of the major routes to incorporate dyestuffs in our biological system is by absorption through skin. Some dyes have different degrees of binding ability with protein, collagen and lipids³⁻⁴. Study shows that toxic effect of these synthetic dyes are observed in the presence of the ultraviolet component of sunlight

(285-400 nm)⁵. Photosensitization reactions in our analysis is oxygen dependent in which a photo excited dye transfers energy to molecular oxygen to produce activated oxygen species like singlet oxygen ($^1\text{O}_2$), superoxide radical (HO_2 or (O_2^-)), hydroxyl radical (OH) and hydrogen peroxide. The activated oxygen species contribute to oxidative degradation of protein, enzymes, lipids and other target molecules of cellular systems⁶⁻⁸ and are also responsible to act as tumor promoters in the development of cancer⁹.

In present study, the reason for taking benzanthrone (7*H*-benz(de)anthrance-7-one) is used in the synthesis of many vat and disperse dyes. It has been observed that workers coming into contact with it during manufacture, pulverization and storage develop itching, burning sensation, erythema, roughness, dryness and pigmentation of the skin¹⁰⁻¹¹. Orange II [monoazo, C.I. acid yellow, orange 7 (15510)], a sodium or calcium salt of *p*-(2-hydroxyl-1-naphthalenyl) azo benzenesulfonic acid is used in dyeing of paper, soaps *etc*¹². A blend of orange II with metanil yellow under the trade name to cause deleterious effects in our biological system¹³. Metanil yellow [monoazo, C.I. acid yellow 36 (13065)], a sodium or calcium salt of *m*-(*p*-anilinophenyl-azo) benzenesulfonic acid, is used in the coloring of foodstuffs, soaps, shoe polishes, wood stains, paper staining and cosmetics. *p*-Aminodiphenylamine (*p*-ADPA) and metanilic acid are important metabolites of metanil yellow¹⁴ and are considered to be an active ingredient of metanil yellow induced toxicity¹⁵. *p*-ADPA is used as an intermediate for the synthesis of photographic chemicals, and the manufacture of dyeing reagents¹⁶⁻¹⁷. It is also used as an ingredient in oxidizing types of hair dyes¹⁸. Its properties of skin irritation and sensitization are well known¹⁹.

Experimental

Benzanthrone was obtained from kalyan Dye Stuff Industry, Kalyan, Bombay. Metanil yellow and orange II were obtained from M/s Visco Products Pvt.Ltd., Metanilic acid and nitro blue tetrazolium (NBT) were procured from Sisco Research Lab., Bomaby. 1,4-Diazabicyclo- (2,2,2)- octoane (DABCO), *p*-ADPA, superoxide dismutase (SOD) and *N*, *N*-demethyl-*p*-nitrosoaniline (RNO) were obtained from Sigma ChemicalCo., U.S.A. Sodium azide and histidine (HIS) were obtained from HiMedia Lab., Bombay. Other chemicals and solvents used throughout the study were of analytical grade and obtained from E. Merck- BDH Chemical Company. The dyes were further purified by column chromatography.

Ultraviolet and visible spectra were recorded on a Shimadzu 450 UV-visible spectrophotometer using quartz cells of 5 cm path length. Irradiation experiments were done as follow: 10 mL solutions were taken in a Petri dish (6 cm diameter) and irradiated with direct sunlight. The temperature was maintained at 20 ± 2 °C by using a constant temperature water bath. Irradiation experiments were done in Kanpur, U.P. on sunny days preferably between 9:00 and 11.00 am. The irradiance of emitted light was measured by an International Light IL-730 UV Actinic Radiometer (International Light Company, Newburyport, Mass., U.S.A.) equipped with a calibrated and cosine - corrected ultraviolet radiation detecting probe. Sunlight produced an average ultraviolet radiation of approx. $1.2 \pm 0.1 \times 10^{-2}$ erg. mm⁻². s⁻¹ in the UV-A range (320-400 nm). It also produced approx. $1.0 \pm 0.1 \times 10^{-4}$ erg. mm⁻².s⁻¹ of UV-B (285-320 nm) radiations under similar conditions. The results of intensity measurement were also confirmed by chemical actinometry²⁰. Formation of $^1\text{O}_2$ was determined in aqueous solutions by the method of Kraljic and Mohsini²¹. With water insoluble chemicals, reactions were done in dimethyl formamide (DMF) solution (5-20% in 0.01 M phosphate buffer, pH 7). A 10 mL solution of RNO was prepared in a

phosphate buffer (0.01 M, pH7) and optical density was adjusted between 1.2 and 1.4. Histidine (1×10^{-2} M) was added to the RNO solution as a selective acceptor of $^1\text{O}_2$. Stock solutions (1 mg/mL) of test compounds were prepared in ethanol, DMF or dimethyl sulfoxide (DMSO). RNO and HIS samples were irradiated with various concentrations of test compounds (0.1, 1.0 and $10 \mu\text{g/mL}$) and sunlight dose (0-25 min). Singlet oxygen forms a transannular peroxide intermediate with HIS leading to the bleaching of $^1\text{O}_2$ was also established by examining the reaction kinetics in D_2O and by carrying out quenching studies with sodium azide²² and DABCO²³. Solvents like D_2O and $\text{C}_2\text{H}_5\text{OH}$ are known to prolong the life time of singlet oxygen²⁴. Formation of (O_2^-) was studied by monitoring the photosensitized reduction of NBT²⁵ with some modifications. Reduction of NBT by (O_2^-) leads to the formation of a blue colored product (nitro blue formazan), which is quantitatively estimated spectrophotometrically at 560 nm. A 1.67×10^{-4} M solution of NBT was prepared in carbonate buffer (0.01 M, pH 8) containing 5-20% DMF to keep the reaction product in solution. 10 mL solutions were irradiated with various concentrations of test samples in sunlight for varying lengths of time. Further evidences for the generation of (O_2^-) were obtained by performing quenching studies with SOD²⁶.

Results and Discussion

Singlet oxygen production

A comparative study of the $^1\text{O}_2$ producing ability of various dyes are studied. Only benzathrone was found to generate sufficient $^1\text{O}_2$ to be monitored spectrophotometrically by a drop in optical density at 440 nm due to bleaching of RNO by the transannular peroxide intermediate of HIS and $^1\text{O}_2$. The $^1\text{O}_2$ generating potential of benzathrone was comparable to well known photosensitizing agents like anthracene.

*Singlet oxygen*²⁷

The lowest energy electron configuration of O_2 , which contains two electron in π orbital's, gives rise to three states is given in Table 1.

Table 1. States of O_2 molecule.

State	π_a^*	π_b^*	Energy
$^1\Sigma_g^+$	$\uparrow$	$\downarrow$	155 kJ($\sim 13,000 \text{ cm}^{-1}$)
$^1\Delta_g$	$\uparrow\downarrow$	—	92 kJ($\sim 8,000 \text{ cm}^{-1}$)
$^3\Sigma_g^-$	$\uparrow$	$\downarrow$	0(ground state)

The $^1\Delta_g$ states has a much longer life time than the $^1\Sigma_g^+$ states²⁸. The life time is solvent dependent, varying by order of magnitude in organic solvents. There are two main ways of generating the singlet oxygen molecules: 1 photo chemically by irradiation in presence of sensitizer and 2 chemically. The photochemical route proceeds as follows, where sense represents the photo sensitizer as shown in equation from 1 to 4

Energy transfer from triplet excited $^3\text{sens}^*$ to $^3\text{O}_2$ to give $^1\text{O}_2$ is a spin allowed process .A representative example is the use of excited azoalkane with O_2 ²⁹.

Dose-response relation

The formation of $^1\text{O}_2$ from benzanthrone was found to be both concentration and sunlight dose-dependent. A nearly linear relationship was found between the concentration of benzanthrone (1- 10 $\mu\text{g/mL}$) and the amount of $^1\text{O}_2$ produced after 25 min exposure with sunlight Figure 1. The study was done using a 10 $\mu\text{g/mL}$ concentration of benzanthrone. The order of food stuffs tested against chemical agent for (O_2^-) production are benzanthrone > metanil yellow > *p*- ADPA were found (as shown in Figure 2, 3 & 4).

Figure 1. Dose–response relationship between $^1\text{O}_2$ production and sunlight exposure of benzanthrone 25 min.

Figure 2. O_2^- production by photosensitized reduction of NBT (1.6×10^{-4} M in 0.01 M) carbonate buffer with 5-20% DMF, pH 8 using 10 $\mu\text{g/mL}$ of the metanil yellow (reaction time 12 min.)

Figure 3. $O_2^{\bullet -}$ production by photosensitized reduction of NBT (1.6×10^{-4} M in 0.01 M) carbonate buffer with 5-20% DMF, pH 8 using 10 $\mu\text{g/mL}$ of the *p*-ADPA (reaction time 12 min.)

Figure 4. $O_2^{\bullet -}$ production by photosensitized reduction of NBT (1.6×10^{-4} M in 0.01 M) carbonate buffer with 5-20% DMF, pH 8 using 10 $\mu\text{g/mL}$ of benzanthrone (reaction time 12 min.)

Superoxide radical's production

There are several low lying singlet states that are important in photochemical oxidation .Like NO ,which has one unpaired electron in an antibonding (π^*)MO ,oxygen molecules associate only weakly ,and true electron pairing to form a symmetrical O_4 species apparently does not occur even in solid. Both liquid and solid O_2 are pale blue. The comparative study of the various bond values for dioxygen species is summarized in Table 2.

Table 2. Various bond values for dioxygen species.

Species	O-O Distance, Å	Number of π^* electron	V_{∞} , cm^{-1}
O_2^+	1.12	1	1905
O_2	1.21	2	1580
O_2^-	1.33	3	1097
O_2^{2-}	1.49	4	802

Chemical property of dioxygens

Oxygen combines with almost all other elements, usually however, only on heating. Dioxygen will some time react reversibly³⁰ with certain metal complexes and their ligand behavior of O_2 . Many studies have been made on the mechanism³¹ of the reduction of O_2 . There is no evidence for four or two electron reduction steps, as would be suggested by the over all reactions from equation no 5 to 9.

The first step is one electron reduction to the superoxide radical ion O_2^- . The potential for reduction

Ranges from ~ -0.2 to -0.5 V depending on the medium. The O_2^- ion is a moderate reducing agent, comparable to dithionite, and a very weak oxidizing agent's. Thus most of the oxidation by O_2^- is due to peroxide ion H O_2^- and O_2^{2-} , formed by reaction such as

The oxidation of water to O_2 in photosynthetic system in plants has been greatly studied³² as have synthetic models such as those of manganese complexes; the oxidation seems occur likely to proceed via H_2O_2 rather than $\text{OH}^\cdot$ Radicals and probably two or four manganese centre are involved³³. It was interesting to know that metanil yellow and *p*-aminodiphenylamine, the two dyes which gave a negative test for $^1\text{O}_2$, were found to produce (O_2^-) in appreciable yields (Figure 2 and 3). Benzanthrone produced (O_2^-) in almost the same quantity as produced by anthracene and hematoporphyrin³⁴ (Figure 4).

Results and Discussion

Selected dyes and dyestuffs, benzanthrone, orange II, metanil yellow and *p*-ADPA most commonly used in colouring and textile industries as well as food additives were examined for their ability to produce activated oxygen species [$^1\text{O}_2$ and (O_2^-)] under normal sunlight with a view to investigating the possible occupational health hazard to people through a series of photodynamic oxidation reactions leading to cell damage.

The formation of $^1\text{O}_2$ by benzanthrone and (O_2^-) by metanil yellow, *p*-ADPA and benzanthrone might rise several question about their safety particularly in conjugation with exposure to sunlight. Studies shows that exposure to metanil yellow, *p*-ADPA and benzanthrone may lead to various short and long term skin photosensitization and cell damaging effects.

Photosensitized reactions of dyes leading to the formation of activated oxygen species are believed to be a matter of great concern because the later are involved in the destruction of cells, tissues, phagocytes and certain cancer inducing processes^{5, 7, 9, 11}. Moreover, Bickers

*et al*³⁵ have demonstrated the destruction of epidermal cytochrome *P*-450 in epidermal microsomes by $^1\text{O}_2$ generated through photosensitization reactions induced by hematoporphyrin derivatives. Several other studies have also suggested that $^1\text{O}_2$, (O_2^-) and other reactive forms of oxygen play a major role in the UV-induced membrane damaging reaction³⁶⁻³⁷. Although spectroscopic studies of cutaneous photosensitizing agent XVII, benzanthrone were carried out by Reza Dabestani *et al*³⁸. The important roles of reactive oxygen species in diseases related to aging and the necessity and benefits of antioxidative nutraceuticals in prevention of disease and promotion of healthy aging reported in recent years³⁹⁻⁴¹.

Production of $^1\text{O}_2$ and O_2^- at different temperature

Incubation of reagent mixture used for determining $^1\text{O}_2$ and O_2^- in the presence of all the test compounds at 0, 25 and 50 °C did not produce any $^1\text{O}_2$ and O_2^- . The results were in agreement with our concepts that photo excitation is necessary for the generation of $^1\text{O}_2$ and O_2^- species from test compounds under our experimental condition .

Conclusion

Analysis was carried out of above dyes at concentration range of (1-10 µg/mL) and exposure period (0-25 min) for obtaining dose response relationship and (0-12 min) for determination of (O_2^-) production of above dyestuffs. At higher concentration the linearity was disturbed due to lack of penetration of solar radiation through the colloidal system.

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