Oxygen Consumption during Photobleaching of Aqueous Solutions of Rose Bengal

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The kinetics of the photodegradation of rose bengal in oxygenated aqueous solutions was studied. The reaction was first order in dye concentration. At O_2/dye ratios of less than 5, the apparent order with respect to oxygen was 0.5. At higher ratios, the apparent order for oxygen approached zero and the reaction became pseudo first order. Since the stoichiometry of the reaction resulted in approximately two molecules of O_2 consumed per molecule of dye degraded, the presence of reaction intermediates was indicated. High-pressure liquid chromatography showed the presence of transient intermediates during the photodegradation.

Rose bengal is one of a number of substituted xanthene dyes (Figure 1) that have been used as laboratory and field toxicants with several insect species (Broome et al., 1975; David and Heitz, 1978; Pimprikar et al., 1980; Carpenter et al., 1981). Laboratory toxicity has been related to the ability of the dyes to form a triplet excited state and to subsequently sensitize the formation of excited singlet oxygen (Callaham et al., 1977). The pesticidal activity of the xanthene series generally increases as the number and atomic weight of the substituent halogens increase. Increased halogen mass generally causes an increase in the relative population of the first excited triplet state of the dye upon illumination by visible light (Gollnick and Schenck, 1964). The increased level of the triplet state of the dye causes an increase in the sensitization of oxygen molecules to the first excited state, generally resulting in an increase in the toxic effects in insects (Callaham et al., 1975; Fondren et al., 1978). The xanthene dyes in aqueous solution are observed to photodegrade when exposed to visible light, and the rate of degradation depends on the oxygen concentration. The need to understand factors that affect the lifetime of the dyes in the environment has led us to study the influence of oxygen on the kinetics of photodegradation of rose bengal.

A comparison of the relative susceptibility to photobleaching of a series of xanthene dyes in aqueous solution has been presented by Heitz and Wilson (1978). Other studies specifically involving xanthene dyes have been performed using flash photolysis (Kasche and Lindqvist, 1964; Zwicker and Grossweiner, 1963) and oxygen consumption experiments (Imamura and Koizumi, 1955; Usui et al., 1965). The oxygen consumption experiments were performed on eosine, erythrosine, and fluorescein and indicated that photobleaching of the dyes was complex in that the reaction mechanism was dependent on dye concentration. For low dye concentrations ($\leq 1.0 \times 10^{-5}$ M) relative to oxygen concentration ($\sim 2.5 \times 10^{-4}$ M in air-saturated solutions) the primary step in photodestruction was an attack on the triplet state of the dye by oxygen (D–O mechanism) leading to decomposition of the dye. Under these conditions, the photobleaching obeyed first-order kinetics. However, as the concentration of the dye became comparable to, or greater than, the oxygen concentration, the first-order kinetics were no longer obeyed. Usui et al. (1965) concluded that there was a switchover from the D–O mechanism at low concentrations of the xanthene dye to a dye–dye (D–D) mechanism at higher dye concentrations in which electron transfer between the triplet-state dye and the ground-state dye became the decisive process. Evidence for the possibility of such an electron transfer has been presented as a result of flash photolytic experiments (Kasche and Lindqvist, 1964). Our experiments with rose bengal have approximated field conditions in that aqueous solutions were studied in which the dye concentration was considerably less than the dissolved oxygen concentration—a situation in which the D–O mechanism should apply. We also report the oxygen consumption for solutions in which the dissolved oxygen concentration was considerably less than the dye concentration, a situation in which the D–D mechanism should apply.

MATERIALS AND METHODS

Rose bengal used in this study was a gift from the Hilton-Davis Chemical Co. and was of certifiable (>85%) grade. A stock solution of the sodium salt of the dye was prepared in deionized water at a concentration of 3×10^{-3} M. Aliquots of the stock solution were diluted with water to concentrations appropriate for each kinetic study. Photobleaching of the dye was accomplished by illumination of the solution with a Dolan-Jenner Model 150 high-intensity white light source. The intensity (irradiance) of the incident light was constant to within ±5% of the average value during the course of any single experiment. Measurements of the irradiance were performed with an EG & G Model 450-1 radiometer/photometer.

In order to study the effects of dissolved oxygen concentration on reaction rate, a closed circulating system shown in Figure 2 was utilized for all irradiations. The dye solution was placed in the syringe, and water-saturated nitrogen or oxygen gas was bubbled through the solution. A peristaltic pump circulated the dye solution through a 10 mm path length special optical glass cylindrical cell from Hellma and back into the syringe via a closed path. The cell was provided with a jacket, which allowed the temperature of the dye solution to be controlled at 25 °C. The dissolved oxygen concentration was measured with a Beckman Model 0269 oxygen analyzer equipped with an oxygen electrode immersed in the solution. When the desired oxygen content was obtained, photodegradation of the dye was performed by irradiating the solution in the cell. The concentration of rose bengal was monitored as a function of illumination time by performing transmittance measurements at 550 nm of the solution in the cell with a Beckman DU spectrophotometer. Alternatively, the dye solution contained in the syringe was illuminated, and the oxygen concentration was monitored with the oxygen analyzer.

High-performance liquid chromatograms of the rose bengal solutions were obtained by using a Waters M6000A pump, a μBondapak reverse-phase C_{18} column, a U6K

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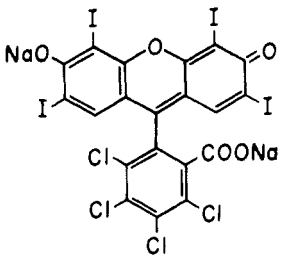

ROSE BENGAL

Figure 1. Molecular structure of rose bengal.

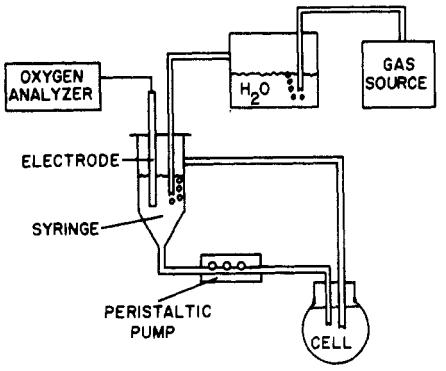

Figure 2. Schematic of the apparatus used for the oxygen consumption experiments.

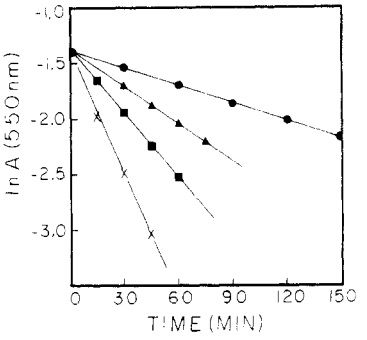

Figure 3. Decrease in absorbance at 550 nm of a rose bengal solution as a function of illumination time for different incident light intensities. The relative intensities were (●) 2.4×10^4, (▲) 4.8×10^4, (■) 8.4×10^4, and (×) 14.4×10^4 $\mu W/cm^2$.

injector, and a Model 440 UV–visible detector, which was set at 546 nm. Samples were eluted with a 70% methanol–30% 0.01 M ammonium acetate buffer, pH 4.0.

RESULTS AND DISCUSSION

A dilute solution (3.3×10^{-6} M) of rose bengal in air-saturated solution ($[O_2] = 2.5 \times 10^{-4}$ M) was photodegraded at various incident light intensities. Figure 3 shows that the decrease in dye concentration follows first-order kinetics and is directly proportional to the light intensity. The dependence of the rate of photodegradation of rose bengal on the dissolved oxygen concentration was studied by utilizing the method of initial rates (Figure 4). The initial rose bengal concentration (3.3×10^{-6} M) as well as the incident light intensity were kept constant during successive experiments, while the initial dissolved oxygen concentration, $[O_2]_i$, was varied. The initial rate of disappearance of rose bengal was strongly dependent on $[O_2]_i$ for oxygen concentrations less than about 3×10^{-5} M O_2. Figure 4 shows that as the $[O_2]_i$ decreased and became comparable to the rose bengal concentration, the reaction rate decreased sharply. Complete deoxygenation of the solution resulted in no detectable photodegradation of the rose bengal. At oxygen concentrations higher than about

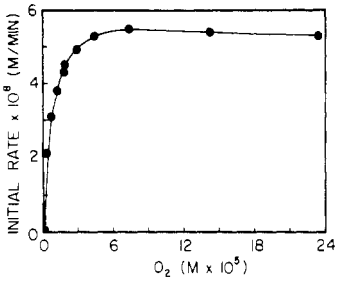

Figure 4. Initial rate of photodegradation of rose bengal (3.3×10^{-6} M) as a function of the initial oxygen concentration.

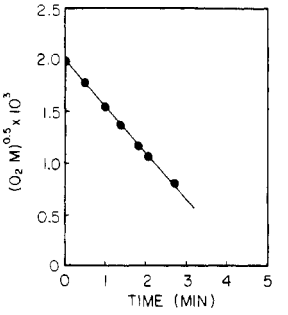

Figure 5. Decrease of oxygen concentration in a rose bengal solution as a function of illumination time.

5×10^{-5} M, the reaction rate became essentially constant. Increasing the initial oxygen concentration beyond that found at room conditions ($\sim 2.5 \times 10^{-4}$ M) actually decreased the reaction rate slightly, presumably because of physical quenching of the triplet dye by oxygen (Kasche and Lindqvist, 1964). At much higher light intensities and under nitrogen atmosphere, Tonogai et al. (1979) have reported that rose bengal photodegrades to tetrachlorofluorescein and erythrosin photodegrades to fluorescein. In the absence of oxygen, it appears that the major degradation involves deiodination of the ring systems. In the presence of oxygen it appears that excited singlet oxygen generation results in a more rapid as well as more complex degradation scheme.

If the rate data from Figure 4 with $[O_2]_i$ less than about 2×10^{-5} M were plotted as log rate vs. log $[O_2]_i$, the resulting least-squares line exhibited a slope equal to 0.54 ± 0.01, giving the apparent order of the photodegradation reaction with respect to oxygen concentration. This result was observed only for the experimental condition that the oxygen concentration was small enough so that the overall reaction was significantly rate limited by the oxygen concentration. This condition was fulfilled when $[O_2]/[$rose bengal$]$ was less than about 5. For higher $[O_2]/[$rose bengal$]$ ratios, the slope of the plot approached zero as the rate became independent of oxygen concentrations.

The oxygen consumption accompanying the photodegradation of rose bengal was monitored by measuring the oxygen concentration as a function of time. The rose bengal solution was placed in the syringe and deoxygenated until the $[O_2]/[$rose bengal$]$ ratio was 0.3. As the solution was illuminated the dissolved oxygen concentration decreased as oxygen was consumed by the photodecomposition. Figure 5 shows that a plot of $[O_2]^{0.5}$ vs. time gave a linear plot. This result was consistent with the apparent reaction order for oxygen obtained from the rate data shown in Figure 4. Similar plots were obtained for $[O_2]/[$rose bengal$]$ ratios of 0.1 and 0.03.

The stoichiometry of the reaction between rose bengal and oxygen was then investigated (Table I). The initial transmittance of a rose bengal solution was measured at 550 nm, and then the solution was deoxygenated so that

Table I. Stoichiometry of Reaction between Rose Bengal and Oxygen

rose bengal, 10^{-5} M	oxygen, 10^{-5} M	[oxygen]/[rose bengal]	Δ[oxygen]/Δ[rose bengal]
33	1.0	0.030	1.1
13	1.0	0.077	1.2
3.3	1.0	0.30	2.4
0.7	1.0	1.4	2.2

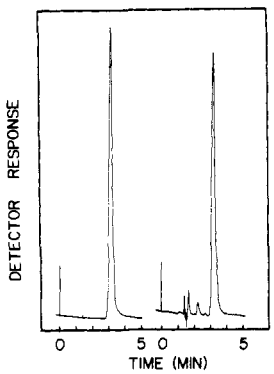

Figure 6. High-performance liquid chromatogram of purified rose bengal (left) and partially photodegraded rose bengal (right) observed at 546 nm.

the $[O_2]$ was about 1×10^{-5} M. The rose bengal concentrations were varied from 6.6×10^{-6} to 3.3×10^{-4} M. The solution was then illuminated and the decrease in oxygen concentration was noted. The final rose bengal concentration was determined by a transmittance measurement. The number of moles of oxygen observed to be consumed for every mole of rose bengal photodegraded, Δ[oxygen]/Δ[rose bengal], increased and reached a value of 2.2 at an [oxygen]/[rose bengal] ratio of 1.4. Values for Δ[oxygen]/Δ[dye] have been reported for eosine and fluorescein (Usui et al., 1965), which were also greater than 2 and increased as the dye concentration decreased.

The observations that the apparent reaction order for oxygen was nonintegral and that the stoichiometric coefficient for oxygen exceeded the reaction order suggested that intermediates were present in the reaction sequence (Edwards et al., 1968). In order to test for the presence of intermediates, rose bengal solutions were subjected to high-performance liquid chromatography (HPLC) before and after partial photodegradation. Figure 6 shows the HPLC trace of purified rose bengal before photodegradation (left side) and indicated that the solution consisted of only one absorbing species at 546 nm. The solution was then illuminated for about 1 min so that the rose bengal was only slightly photodegraded. The HPLC trace (right side) that resulted from this solution showed not only the unreacted rose bengal peak but also several additional peaks. As the solution was illuminated for longer periods of time, the new peaks first increased in amplitude to a maximum and then decreased. A completely photodegraded solution showed no visible absorption at the rose bengal position or for any of the additional peaks that must have been intermediate species in the overall reaction. Intermediates have also been reported (Tonogai et al., 1979) by thin-layer chromatography in a study of the photodecomposition products of rose bengal.

The general reaction scheme for the photodecomposition of other xanthene dyes leads to the following expression for the overall reaction rate (Usui et al., 1965)

$$\text{rate} = -d[O_2]/dt = A[D][O_2]/(B + C[O_2]) \quad (1)$$

where [D] and $[O_2]$ represent the concentration of dye and oxygen and A, B, and C are constants for a particular dye. The form for this equation suggests two limiting values for the reaction order with respect to $[O_2]$. First, if $C[O_2] \gg B$, then the rate expression becomes

$$\text{rate} = A[D]/C \quad (2)$$

independent of $[O_2]$ and the reaction order with respect to $[O_2]$ is zero. On the other and, if $B \gg C[O_2]$, then the rate expression becomes

$$\text{rate} = A[D][O_2]/B \quad (3)$$

or first order with respect to $[O_2]$. At a condition for which neither term in the denominator is dominant, the observed overall reaction order with respect to $[O_2]$ will lie somewhere between zero and one, depending on the relative magnitude of the terms B and $C[O_2]$. For our experimental conditions, the measured value was 0.54.

The reaction scheme leading to eq 1 also predicts that Δ[oxygen]/Δ[rose bengal] should be unity, and our data approach this value at low [oxygen]/[rose bengal] ratios, but as the relative concentration of oxygen increases, the Δ[oxygen]/Δ[rose bengal] becomes significantly larger than unity. This observation may lead to a search for an oxygen-consuming reaction in addition to those in the primary bleaching process, since one possibility is that the reaction intermediates also react with oxygen as photodegradation proceeds. In this paper the photodegradation process has been studied by an integrated design involving observation of dye concentrations by spectrophotometry and oxygen concentrations and by observation of dye degradation intermediates by HPLC. Future studies may require an expansion from this protocol to include observation of changes in pH and halogen ion production for a more complete understanding of the photobleaching process.

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