A Study of the Superoxide Radical Chemistry by Stopped-Flow Radiolysis and Radiation Induced Oxygen Consumption

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Abstract: The chemical reactivity of the superoxide radical has been studied at pH 10 and 23 °C by absorption spectroscopy in a fast kinetics spectrophotometer and by radiation induced oxygen consumption in a modified stopped-flow radiolysis apparatus on line with a Van de Graaff electron generator. The latter method differentiates between oxidation and reduction reactions and yields information on the corresponding stoichiometry. A number of examples are used to demonstrate the versatility of these techniques. Rate constants were determined for the reaction of superoxide radicals with: ferricytochrome *c*, $k = 2.6 \pm 0.2 \times 10^5$ M^{-1} s^{-1} at pH 9.0; ascorbate, $k = 1.52 \pm 0.1 \times 10^5$ M^{-1} s^{-1} at pH 9.9; nitroblue tetrazolium, $k = 5.94 \pm 0.5 \times 10^4$ M^{-1} s^{-1} at pH 9.8. Some 19 other compounds (buffers, carboxylic acids, chelating agents, etc.) were shown to be inert toward superoxide radicals.

The finding of a superoxide radical dismutation activity in bovine erythrocuprein,[1] which was therefore named superoxide dismutase, initiated one of the most intense research efforts on a single chemical species (O_2^-) in recent years. The results of these efforts, particularly those which have led to clinical applications, as well as the unresolved problems and controversies have been reviewed recently in several articles.[2-5]

The mechanisms of involvement of the superoxide radical in many biological systems are not yet well understood. Often conclusions are based upon indirect experimental observations and assumed O_2^- reactivities which have not been verified.[3]

Although there is some thermodynamic information available from which one can compute whether O_2^- or HO_2 can react with a particular compound,[6] such predictions are not always possible or accurate since frequently little is known about the degree of resonance stabilization of the product radical. This is particularly true in biological systems where an unstable radical could be stabilized on an enzyme,[7] membrane, or some other species.

The objective of the project discussed in this report was to initiate the development of techniques which would permit the rapid evaluation of the chemical reactivity of superoxide radicals with compounds of biological interest. This report describes two such techniques, one of which, that is, the stopped-flow method, has been described briefly in an earlier publication.[8] Very recently a stopped-flow method has been reported in which an aprotic solvent containing stabilized O_2^- is mixed with aqueous solutions.[9]

A stopped-flow radiolysis technique has been developed for spectrophotometric studies of secondary free radicals which have relatively long lifetimes, that is, half-lives in excess of a few milliseconds. This new method, which has the advantages and limitations of ordinary stopped-flow techniques,[10] offers the opportunity to study free-radical reactions in isolation; that is, a single radical species can be studied in absence of other radicals. This advantage is particularly important in studies of radicals which react slowly with scavengers, as the high substrate concentrations which are required would lead to unwanted side reactions if other radicals are present. In the stopped-flow radiolysis technique all primary radicals formed from the irradiation of water (reaction I) decay or are converted to a secondary radical species, e.g., O_2^- in oxygenated formate solutions (reactions 1–5), before the flowing solution reaches the mixer where it encounters the scavenger under study.

An alternate method has been developed, namely, a modified stopped-flow radiolysis technique,[11] which in the present state of development permits an approximate evaluation of the rate of a reaction of compounds with superoxide radicals. The method is based upon the consumption of molecular oxygen in a given system and has the advantage of being applicable in cases where spectrophotometry fails. Also, this method could be developed for nonaqueous systems as well as for solution mixtures in which other analytical methods cannot be utilized.

Small traces of heavy metals can act as catalysts in O_2^- reactions.[12,13] It is necessary that all additives required in the experiments, such as buffers, salts, and chelating agents, not only fail to react with O_2^- themselves but are free of impurities which could affect the reactions studied.

Since this part of the overall program is aimed at the study of the superoxide ion chemistry only, the experiments were carried out at pH 10. At this pH many compounds are relatively stable and interference from the HO_2 radical is minimized (based upon $pK_{HO_2} = 4.8 \pm 0.1$, $(HO_2) = 5 \times 10^{-6}(O_2^-)$).[14,15] Interference by HO_2 is easily checked by lowering the pH while all other experimental conditions are kept constant. Also, since the first half-life for spontaneous disproportionation of O_2^- is relatively long (our observation 70–100 s) for the superoxide radical concentrations used in these experiments, relatively slow rate processes for its reaction with scavengers can be determined.

Experimental Section

Chemicals and Solutions. The water used for all phases of work in this study was first triply distilled from acidic dichromate and alkaline permanganate and then redistilled from 1 mM ethylenediaminetetraacetate (EDTA) in an all-silica distilling apparatus. The latter was provided with a flash evaporator which prevented the liquid surface film from creeping into the condenser. The water was collected, stored, and handled in silica vessels only.

Commercial compounds which needed purification were recrystallized three times from this purified water in presence of 0.1 mM EDTA (ethylenediaminetetraacetic acid). The latter was added for effective removal of heavy metal impurities. A final recrystallization was always carried out in absence of EDTA. Since commercially available EDTA contains about 0.001% iron and 0.001% heavy metals, it was always recrystallized several times before use.

The following chemicals were purified by the above method before use: sodium carbonate, sodium formate, mono-, di-, and trisodium phosphate (Baker Analyzed reagent); ethylenediaminetetraacetate (Fisher Scientific Co.).

Compounds used in this study without further purification were sodium acetate, citric acid (Baker Analyzed reagent); sodium lactate, sodium malate, maleic acid, tris(hydroxymethyl)aminoethane, potassium fumarate, potassium pyruvate (Sigma Chemical Co.); sodium succinate, oxalic acid (Fisher Scientific Co.); sodium borate, potas-

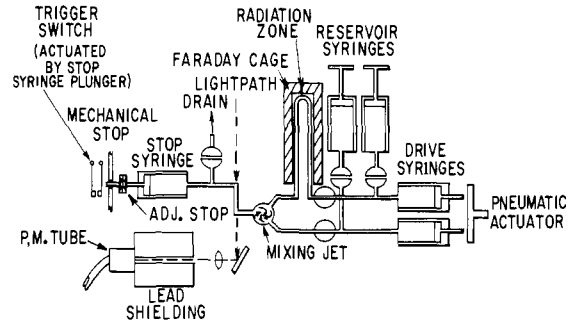

Figure 1. Fast kinetics spectrophotometer.

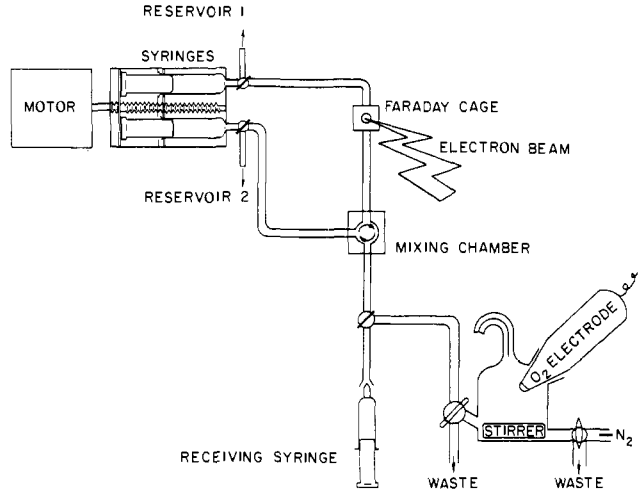

Figure 2. Continuous flow-radiolysis apparatus.

sium tartrate (Baker & Adamson, Allied Chemical Corp.); α-ketoglutaric acid (Boehringer Mannheim); imidazole, 1-methylimidazole, 2-methylimidazole (Aldrich Chemical Co. Inc.); perchloric acid, double vacuum distilled from Vycor (G. Frederick Smith Chemical Co.).

The purity of the chemicals and water was tested after each stage of purification by comparing the half-life times of O_2^- decay. For water and other nonreactive chemicals, the half-lives of O_2^- increase with increasing purity of the given system. The adopted standard is that if $t_{1/2}$ does not increase after two successive purifications (distillations or recrystallizations), the compound is considered pure. This way it was established that EDTA, sodium formate, and trisodium phosphate did not improve in purity after three recrystallizations. Similarly the quality of the water did not improve after one redistillation from EDTA in the all-silica distilling apparatus.

The purity of chemicals which do react with O_2^- was tested in a similar manner. A given compound was purified until a constant value was obtained for the half-life time of its interaction with O_2^- under pseudo-first-order conditions.

The pH of all solutions was adjusted by addition of either double vacuum distilled perchloric acid (content of heavy metals 0.000001%, of iron 0.000001%) or three times recrystallized trisodium phosphate.

Apparatus and Methods. Stopped-Flow Radiolysis Technique. A fast kinetics spectrophotometer (Durrum Instrument Co., Model D 110) has been modified so that one of the flowing solutions passes through a 2-MeV electron beam produced by a Van de Graaff generator. A solution of the radical of interest is generated as the liquid passes through the electron beam, and prior to mixing with the second flowing solution all primary radicals have decayed or been converted to the radical of interest. The nonirradiated solution carries the scavenger and/or buffer at the desired pH (Figure 1).

The inverted U-shaped irradiation tube is approximately 20 cm long (10 cm from bend to fitting) and has an inner diameter of 0.2 cm. Hence in order that a homogeneously irradiated solution fills the entire flow track past the optical cell after mixing with the scavenger solution, a minimum volume of about 0.3 mL has to be irradiated. This condition is easily satisfied by setting the stop mechanism for a total displacement of 0.5 mL from each syringe and by moving the radiation zone as close to the mixing chamber as is practical. It was found that excellent results were obtained with a defocused electron beam which gave at the flow tube (which was 15 cm from the Van de Graaff window) a homogeneous radiation zone of approximately 2 cm in diameter. Under these conditions free-radical concentrations of up to 30 μM were generated with a continuous 2-MeV electron beam.

The spectrophotometer covers the spectral range from 200 to 800 nm and can be used with a 1, 2, or 5 cm optical light path. The dead-time between the radiation zone and the mixer is from 8 ms upward depending upon the location at which the flow tube is being irradiated. The dead-time between mixer and optical cell is of the order of 2 ms.

Radiation-Induced Oxygen Consumption Method. The continuous flow-radiolysis apparatus shown in Figure 2 can be operated with either two or four 50-mL syringes. The beginning of the flow and of the irradiation with a continuous electron beam from a Van de Graaff generator is electronically synchronized. When the receiving syringe is filled the operation is automatically terminated. In this setup, superoxide radical concentrations up to 100 μM are generated in the flow tube located 2.5 cm from the Van de Graaff window. The solution containing the O_2^- radicals is rapidly mixed in a jet-mixer with a buffer solution containing the other reactant. The solution mixture is subsequently collected in a receiving syringe for oxygen analysis with a YSI 5750 oxygen electrode and a YSI Model 57 Oxygen Meter (Yellow Springs Instrument Co.). A 15-mL chamber holding the oxygen electrode and a magnetic stirring bar was connected to the receiving syringe of the continuous-flow apparatus. For good reproducibility the chamber was flushed with UHP nitrogen before a given sample was injected for analysis. Reproducible readings were obtained within 2–3 min.

The limit of the sensitivity of this oxygen electrode is of the order of 3 μM. In most of these experiments air-saturated solutions were used, so that before irradiation $[O_2] = 250$ μM. Thus, a change of less than 1% in the initial oxygen level can be detected. In general, initial concentrations of O_2^- of about 50 μM were generated (20% conversion of the initial O_2), of which in absence of a reactive scavenger 50% was reconverted to O_2. Therefore in a typical experiment a 10% decrease in $[O_2]$ occurred. This corresponds in terms of G values to $G(-O_2) = 3.2 \pm 0.4$ for the solutions used.

The amount of superoxide radicals formed in a given run was computed from a ferrous dosimeter calibration curve, which was determined by running a ferrous sulfate solution in the flow apparatus and measuring the radiation induced formation of ferric iron as a function of energy input. The superoxide radical concentrations were computed on basis of $G(O_2^-) = 6.4$ and $G(Fe^{3+}) = 15.5$.[16]

Results

Superoxide free radicals were generated by irradiation of air-saturated sodium formate solutions with 2-MeV electrons. The mechanism by which O_2^- is generated in formate solutions has been studied extensively and is well established.[17] It is described by the following set of equations, which prescribes an observed yield of $G(O_2^-) = G(HO + H + e_{aq}^-) = 6.1$ (G = number of free radicals generated per 100 eV of energy absorbed):[18]

$$H_2O \rightsquigarrow H, HO, e_{aq}^-, H_3O^+, H_2O_2, H_2 \quad (1)$$

$$H + O_2 \rightarrow HO_2 \quad (1)$$

$$e_{aq}^- + O_2 \rightarrow O_2^- \quad (2)$$

$$HO_2 \rightleftharpoons O_2^- + H^+ \quad (3,-3)$$

$$HCOO^- + HO \rightarrow COO^- + H_2O \quad (4)$$

$$COO^- + O_2 \rightarrow O_2^- + CO_2 \quad (5)$$

As a result of extensive preliminary studies, a procedure was adopted by which O_2^- radicals were always generated in an air-saturated 0.2 M sodium formate solution containing 0.2 mM EDTA and adjusted to pH 10 with trisodium phosphate.

It was established by stopped-flow experiments that formate

Table I

Compd	Concn, M	pH	k_6, M^{-1} s^{-1}	$G(-O_2)^a$
(1) Acetate	0.0100–0.10	10.1	<0.06	3.6 ± 0.4
(2) Borate	0.0100–0.10	10.0	<0.02	3.1 ± 0.3
(3) Carbonate/bicarbonate	0.0100–0.25	10.1	<0.04	3.6 ± 0.2
(4) Citrate	0.0100–0.10	10.1	<0.14	3.3 ± 0.1
(5) Ethylenediaminetetraacetate (EDTA)	0.0100–0.10	9.9	<0.01	3.4 ± 0.4
(6) Formate	0.0005–1.00	10.1	<0.01	3.2 ± 0.2
(7) Fumarate	0.0100–0.10	10.1	<0.10	3.4 ± 0.2
(8) Imidazole	0.0100–0.10	10.1	<0.02	3.4 ± 0.2
(9) Imidazole (1-methyl-)	0.0100–0.10	10.1	<0.15	3.2 ± 0.2
(10) Imidazole (2-methyl-)	0.0100–0.10	10.1	<0.18	3.5 ± 0.2
(11) α-Ketoglutarate	0.0100–0.02	10.1	<0.30	3.2 ± 0.2
(12) L-Lactate	0.0100–0.10	10.0	<0.50	3.3 ± 0.2
(13) L-Malate	0.0100–0.10	10.1	<0.11	3.5 ± 0.2
(14) Maleate	0.0001–0.05	10.0	<0.06	3.3 ± 0.1
(15) Oxalate	0.0050–0.05	10.0	<0.20	3.5 ± 0.2
(16) Pyruvate	0.0010–0.01	10.0	<0.10	3.3 ± 0.1
(17) Succinate	0.0100–0.10	9.9	<0.25	3.5 ± 0.2
(18) Tartrate	0.0100–0.10	10.1	<0.14	3.7 ± 0.1
(19) Tris(hydroxymethyl)aminomethane	0.0100–0.10	10.1	<0.001	3.3 ± 0.4

[a] All oxygen determinations were carried out at the highest scavenger concentration listed except the formate system which was studied in 0.1 M solution.

effectively protects EDTA from OH radical attack when the ratio of $(HCOO^-)/(EDTA) > 100$. In a series of duplicate experiments the total yield of O_2^- formed at constant energy input was compared for formate solutions irradiated in the presence and absence of EDTA. The results showed no difference within experimental error in the yield for the two different experimental runs. This finding is consistent with the reported rate constant for the reaction of OH with $HCOO^-$ ($k = 3.5 \times 10^9$ M^{-1} s^{-1})[19] and for OH with EDTA ($k = 2.8 \times 10^9$ M^{-1} s^{-1}).[20]

Spectrophotometric Stopped-Flow Radiolysis Studies. Spectrophotometric studies of the reactivity of superoxide radicals with other compounds by the stopped-flow radiolysis method were routinely started with the determination of the spontaneous radical decay rate, which served as a correction factor for rate studies with scavengers. Although the absorption maximum for O_2^- is at 245 nm,[14,15] our measurements were carried out at 250 nm because of a more favorable signal-to-noise ratio. Reactants or reaction products with relatively strong absorbing bands (which interfere with measurements in the 250-nm region) were studied at their corresponding optical maxima.

A. Ferricytochrome c. The reduction of ferricytochrome *c* by O_2^- was monitored at 550 nm, The reaction was studied under pseudo-first-order conditions over a cytochrome *c* concentration range from 0.2 to 0.05 mM after mixing, The observed rate constant $k = 2.6 \pm 0.2 \times 10^5$ M^{-1} s^{-1} at pH 9.0 is somewhat higher than similar rate constants reported earlier.[21-24] The most likely reason for this discrepancy is that above pH 7.4 cytochrome *c* is known to be present in two different forms which equilibrate at pH 9.0 over a period of many seconds.[25,26] In the present investigation cytochrome *c* was always kept at pH 5.8 before mixing with O_2^- at pH 9.0. Since the overall reduction of ferricytochrome *c* by O_2^- was completed in less than 0.2 s after mixing, one can assume that no significant configurational changes took place during this short time period and hence that our observed rate constant represents the configuration present below pH 7.4. The observed rate constant thus agrees with the previously reported higher rates of reaction of ferricytochrome *c* with O_2^- at lower pH values.[23]

B. Ascorbic Acid. The reaction of superoxide radicals with ascorbic acid was studied at pH 9.9. The reaction was monitored at 270 nm, the absorption maximum of ascorbate (AH^-). In order to avoid spontaneous air oxidation of ascorbic acid, UHP nitrogen saturated solutions of the latter were kept at pH 4.2. Control experiments monitoring the spontaneous air oxidation of ascorbate at pH 9.9 ($k \sim 2.0$ M^{-1} s^{-1}) were carried out by mixing the nitrogen saturated ascorbate solutions with the nonirradiated formate–EDTA–buffer solution. A second-order rate constant $k = 1.52 \pm 0.1 \times 10^5$ M^{-1} s^{-1} was computed for the interaction of O_2^- with AH^-. This value was obtained from pseudo-first-order decay rates for an AH^- concentration range from 10 to 50 μM. A similar rate constant, $k = 2.7 \times 10^5$ M^{-1} s^{-1}, was reported earlier for pH 7.4.[27]

C. Nitroblue Tetrazolium (NBT). The reduction of nitroblue tetrazolium to the blue formazan[28-31] is frequently used as evidence for the generation of O_2^- in biological systems. A second-order rate constant, $k = 5.94 \times 10^4$ M^{-1} s^{-1}, was obtained from pseudo-first-order rate measurements at 560 nm for a NBT concentration range from 0.25 to 1.0 mM at pH 9.8.

D. Transparent Compounds. The reactivity of the superoxide radical toward a number of compounds X which are either transparent or have a very low absorbance between 220 and 280 nm was studied by following the O_2^- absorbance. The O_2^- decay followed second-order kinetics for at least 3 half-lives in the absence of X. In the presence of large amounts of X, the kinetics became mixed first and second order, the first half-life being somewhat smaller than in the absence of X. Rate constants for

$$O_2^- + X \rightarrow \text{product(s)} \qquad (6)$$

were computed by

$$k_6 = \frac{0.693}{[X]}\left[1/(t_{1/2})_{run} - 1/(t_{1/2})_{control}\right] \qquad (II)$$

and are given in Table I. The rate constants are maximum values as the effects observed are quite likely due to impurities. It will be shown later on that values of k_6 computed from spectral decay curves of O_2^- are valid only if reaction 6 is not part of a chain reaction (see case IV).

All experiments were carried out in presence of 0.1 mM EDTA. A further increase in EDTA did not affect the observed O_2^- decay. In absence of EDTA the observed O_2^- decay was always higher and often not reproducible if the solutions were prepared from different batches of chemicals. The latter

finding indicates the presence of catalytic impurities which are most likely heavy metal ions.

Radiation Induced Oxygen Consumption Studies. The above examples illustrate that the reactivity of O_2^- can be studied spectrophotometrically by either observing the absorbance changes of the reactant and/or product, or in transparent systems (230–280 nm) by following the absorbance of the superoxide radical itself. While the first case yields information right away as to the type of reaction, the second case can be complex since it is impossible to judge from O_2^- disappearance measurements alone whether the superoxide radical undergoes oxidation or reduction or whether in a given system its observed rate of disappearance is superimposed upon a fast chain reaction.

To overcome this difficulty, we developed the radiation induced oxygen consumption method outlined in the Experimental Section. While oxygen concentration measurements are fast and standard for most aqueous solutions, determination of concentration changes in reactants or products which are optically transparent can be very tedious. The oxygen method is based upon the quantitative relationship between the initial amount of oxygen consumed in the radiation induced formation of O_2^- and the total amount of oxygen consumed in the system after O_2^- has disappeared in a chemical reaction(s).

Results for the possible types of reactions are:

Case I. The superoxide radical disproportionates to yield back one-half of the molecular oxygen consumed in the radiation induced process of its formation (reactions 1, 1–5. Disproportionation of O_2^- occurs in absence of scavengers;

$$O_2^- + HO_2 + H^+ \rightarrow H_2O_2 + O_2 \qquad (7)$$

and $G(-O_2) = \frac{1}{2}G(O_2^-)$. Under our experimental conditions, that is, in presence of 0.1 M HCOONa, $G(-O_2) = 3.2$.

As can be seen in Table I, the $G(-O_2)$ values determined in the presence of a select number of compounds are within experimental error equal to $\frac{1}{2}G(O_2^-)$ which indicates that O_2^- is disappearing mainly by reacting with itself in reaction 7. Nevertheless, since the superoxide radical has a very long lifetime at pH 10 (under our experimental conditions the first $t_{1/2} \sim 70$ s), it may also react very slowly with the added compound X in reaction 6.

Case II. When the superoxide radical is oxidized, as for example in reactions with ferricytochrome *c* or nitroblue tetrazolium, theoretically $G(-O_2)$ is zero;

$$\text{Cyt } c(\text{Fe III}) + O_2^- \rightarrow \text{Cyt } c(\text{Fe II}) + O_2 \qquad (8)$$

$$NBT + O_2^- \rightarrow NBT^- + O_2 \qquad (9)$$

The experimentally observed $G(-O_2) = 0.1$ is within the limit of the sensitivity of the method and indicates that a very small amount of O_2^- decayed during the flow between the radiation zone and the mixer.

Case III. If the superoxide is reduced by an electron donor (QH_2) to hydrogen peroxide $G(-O_2) = G(O_2^-) = 6.1$;

$$QH^- + O_2^- \xrightarrow{H^+} Q^-\cdot + H_2O_2 \qquad (10)$$

This mechanism holds true only if the product radical, $Q^-\cdot$, does not react with molecular oxygen

$$Q^-\cdot + O_2 \rightarrow Q + O_2^- \qquad (11)$$

or if the rate of $Q^-\cdot$ disproportionation is greater than the rate of reaction 11 ($k_{12} \gg k_{11}$) and appropriate experimental conditions are chosen to minimize reaction 11:

$$Q^-\cdot + Q^-\cdot \xrightarrow{H^+} Q + QH^- \qquad (12)$$

If reaction 11 does occur and at a rapid rate, a chain reaction is initiated which is being discussed in the next case.

Case IV. If the superoxide radicals induce a chain reaction, then $G(-O_2) > G(O_2^-)$. We assume a mechanism

$$O_2^- + RH_2 \xrightarrow{H^+} RH\cdot + H_2O_2 \qquad (13)$$

$$RH\cdot + O_2 \rightarrow O_2^- + \text{product(s)} \qquad (14)$$

$$O_2^- \xrightarrow{H^+} \tfrac{1}{2}O_2 + \tfrac{1}{2}H_2O_2 \qquad (15)$$

$$RH\cdot + RH\cdot \rightarrow \text{product(s)} \qquad (16)$$

Reaction 15 is written as a first-order reaction in spite of the fact that mixed kinetics are observed for O_2^- decay in the presence of large quantities of RH_2. This is an approximation, but the error introduced is small and the complexity of using mixed first- and second-order kinetics is not warranted by the data.

The objective here is the evaluation of an upper limit for k_{13} from the radiation induced oxygen consumption. In the case where $k_{14}[O_2] \gg k_{13}[RH_2]$ and reaction 16 is negligible compared to reaction 14, a steady state exists for RH· and

$$(O_2^-) = (O_2^-)_0 e^{-k_{15}t} \qquad (III)$$

Also within the limits of the above assumptions the change in molecular oxygen concentration after mixing is described by

$$-\frac{d(O_2)}{dt} = [k_{13}(RH_2) - \tfrac{1}{2}k_{15}](O_2^-) \qquad (IV)$$

which upon integration yields

$$\Delta(O_2) = \left[\frac{k_{13}}{k_{15}}(RH_2) + \frac{1}{2}\right](O_2^-)_0 \qquad (V)$$

where $\Delta D(O_2^-)$ is the $(O_2)_{\text{measured}}$ about 5 min after irradiation (infinite time) less the O_2 concentration before irradiation.

Equation V can also be expressed in terms of *G* values, where $G(O_2^-)$ and $G(-O_2)$ are proportional to $(O_2^-)_0$ and $-\Delta(O_2)$, respectively:

$$k_{13} = \frac{k_{15}}{(RH_2)}\left[\frac{G(-O_2)}{G(O_2^-)} - 0.5\right] \qquad (VI)$$

The $G(-O_2)$ values listed in column 5 (Table I) are all within experimental error equal to $\frac{1}{2}G(O_2^-)$, thus proving not only the absence of a chain reaction but also indicating the inertness of these compounds toward superoxide radicals. As is apparent, the information obtained from the radiation induced oxygen consumption method is a valuable addition to the spectrophotometric techniques, since in addition to the overall stoichiometry, it also gives unambiguous information as to the direction in which electrons are transferred. The oxygen consumption predicted for the various modes of reaction of superoxide radicals does not include possible reactions of its reaction product the singlet molecular oxygen ($^1O_2$).[32-34] This aspect will be checked with compounds known to react with $^1O_2$.

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Quenching of the $^1n,\pi^*$ of Alkanones by Unsaturated Compounds[1]

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Abstract: Structure-reactivity profiles of a number of unsaturated compounds toward quenching of alkanone fluorescence were investigated. These studies reveal that the rate constant for fluorescence quenching is sensitive to the electron-donating ability of the dienes and olefins used and to steric effects of the alkanone. The efficiencies of quenching of alkanone fluorescence by a number of unsaturated compounds increase relative to the rates of diffusion in solution as temperature decreases. These results together with other reports in the literature concerning oxetane formation and fluorescence quenching indicate that exciplexes are intermediate in these processes.

I. Introduction

In 1961, Hammond and co-workers reported that the photosensitized cis–trans isomerization of 1,3-pentadienes was a potential probe for understanding many photochemical mechanisms.[3] Evidence was soon forthcoming that the 1,3-diene system was a convenient and well-behaved diffusion controlled quencher of many triplet states ($E_T > 60$ kcal).[4] It was generally assumed that (a) singlet quenching of the sensitizer by 1,3-diene was negligible and (b) irreversible sensitizer–diene chemistry was unimportant.[5] Subsequent careful studies by Hammond[6] and by Schenk[7] showed that neither a nor b was general. However, it was thought that the original generalizations held for alkanones and aryl ketones. In the latter case, singlet quenching by dienes is insignificant because of the normally rapid rate of intersystem crossing ($k_{st} > 10^{10}$ s^{-1}).[8] One expects, however, that the rule of no singlet quenching may break down when k_{st} drops to values less than 10^{10} s^{-1}. It is interesting to note that Barltrop has shown that aryl ketone triplets do form adducts with 1,3-dienes but in very low quantum efficiencies.[9]

1,3-Dienes have been used extensively as quenchers in the study of the mechanisms of alkanone photochemistry.[10] It was supposed that 1,3-dienes were not effective quenchers of alkanone singlets in solution, possibly on the basis of gas-phase measurements which indicated that alkanone fluorescence is not decreased significantly by 1,3-dienes.[11] The elegant use of this idea by Hammond and Wagner[12] to separate singlet and triplet efficiencies and to determine triplet reactivity stimulated considerable use of 1,3-dienes as "triplet specific" quenchers of alkanones.

More recently, however, the quantitative measurements of quenching of alkanone fluorescence by 1,3-dienes left no doubt that significant deactivation of singlet alkanones can be induced by 1,3-dienes.[13] Although many of the qualitative conclusions reached earlier, with the assumption of negligible singlet quenching, are valid, recent quantitative studies suggest that both alkanone and diene structures will determine the rate constant for singlet quenching. We report here a study of the structure–reactivity relationship for quenching of alkanone singlets by 1,3-dienes and olefins, a temperature dependence investigation of the quenching efficiencies of both electron-rich and electron-deficient olefins on alkanone fluorescence, as well as an investigation of the oxetane formation from photoexcited acetone and 1,3-dienes. Results indicate that exciplexes are intermediate in these processes.

II. Results

Table I summarizes our results from the measurement of the rate constants for quenching of alkanone fluorescence by 1,3-dienes. The rate constants for fluorescence quenching, k_q^f, were determined by measuring the decrease in fluorescence intensity or lifetime of the alkanones as a function of diene