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Mechanisms of Photosensitized Oxidation

There are several different types of photosensitized oxidation which may be important in biological systems.

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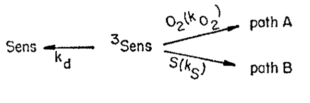
Photosensitized oxidations have been of interest to chemists and biologists since Raab's discovery that microorganisms are killed by light in the presence of oxygen and sensitizing dyes (*1*). These conditions cause pathological effects (referred to as "photodynamic action") in many organisms, including the human. The effects include cell damage, induction of mutations or cancer, and death, and are a consequence of the photooxidation of sensitive cell constituents. Both in vivo and in vitro, nucleic acids are damaged (largely by oxidation of guanine residues), enzymes are deactivated, and polypeptides and proteins are damaged (*2*). Also, carbohydrates are degraded (*3*). Closely related nonpathological photosensitized oxidation-reduction processes are important in photosynthesis (*4*).

Recent studies on simple chemical systems have led to rapid advances in the understanding of photosensitized oxidations. In particular, not one pathway but a number of different ones have been shown to occur. The path actually followed in a given system is a sensitive function of the conditions; some of the factors governing the pathway are discussed in this article. Coverage of the literature is highly selective, rather than exhaustive.

With few exceptions, photosensitized oxidations proceed by way of the triplet sensitizer (Sens) (*5*), probably because the triplet has a much greater lifetime than the excited singlet. The most effective sensitizers are, therefore, those which give a long-lived triplet state in high quantum yield:

$$\text{Sens} \xrightarrow[h\nu]{} {}^1\text{Sens} \rightarrow {}^3\text{Sens} \rightarrow \text{reaction}$$

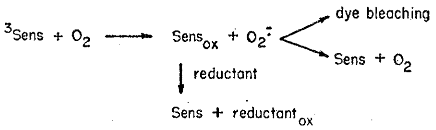
Because of limitations of space, I consider only photosensitized oxidations which occur in the presence of oxygen (aerobic photooxidations). Within this category there are two types of reaction, (i) those in which the primary interaction of the sensitizer triplet is with oxygen and (ii) those in which the interaction is with the substrate (S). The efficiency of each path depends on the relative values of the oxygen and substrate concentrations, the rates of reaction of sensitizer triplet with substrate (k_S) and with oxygen (k_{O_2}), and the rate of triplet decay (k_d). The efficiencies of the two paths can be lowered by various deactivation processes at subsequent stages of the reaction, and are limited by the quantum yield of triplet sensitizer formation (ϕ_T).



In oxygen- or air-saturated solutions, oxygen concentration is in the range of 10^{-2} to $10^{-3}M$ (*6*). Since k_{O_2} is invariably close to 10^9M^{-1} sec^{-1} (*7*), path A will predominate unless the product k_S [S] is on the order of 10^6 to 10^7 sec^{-1}. Conversely, even traces of oxygen will inhibit path B if k_S or S is small. Two examples will illustrate the relationships. For benzophenone in pure isopropyl alcohol (as substrate, 13*M*) under O_2 ($10^{-2}M$), the ratio k_{O_2}/k_S is about 10^3 (*8*), $k_{O_2}[O_2]/k_S[S]$ is about 1, and path A and path B are occurring in roughly equal proportions. However, in the absence of certain specific reactants (discussed below), path A leads only to quenching of sensitizer triplet, and no permanent chemical reaction occurs. Under these conditions path B is only partly inhibited. By contrast, for eosin in ethanol as substrate (and for many other dyes in alcohols), it can be calculated (*7, 9*) that k_{O_2}/k_S is about 10^7 (10^4 higher than the ratio for benzophenone). Thus, even at low oxygen pressures, path B is almost completely inhibited; only in the presence of substrates which are much more reactive than alcohols does any reaction occur by path B with dyes in the presence of oxygen.

Sensitizer-Oxygen Interaction

Two principal interactions of triplet sensitizer with oxygen occur. Flash spectroscopic studies have shown that electron transfer from triplet fluorescein and eosin to oxygen occurs on a small (1 to 10 percent or less) fraction of deactivating encounters (*9, 10*). This reaction apparently leads primarily to oxidative bleaching of the dye in very low quantum yield, and, in part at least, to quenching by recombination of the ion radicals. This route can lead to indirect reactions of the type discussed in the next section by subsequent reaction of oxidized sensitizer with a reductant. (In this and subsequent discussion, the subscripts "ox" and "red" refer to oxidized or reduced forms, usually the radical cation or anion, respectively, or species derived from these by proton loss or capture.)



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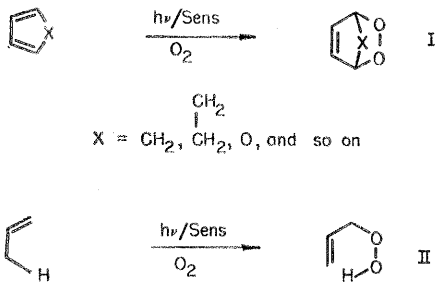


Fig. 1.

A process of electron transfer to oxygen, followed by further reactions, was proposed by Weiss as a general mechanism for dye-sensitized photooxidations (*11*); however, it is probably not important under most conditions because of its low efficiency.

In the presence of suitable acceptors, sensitizers (dyes and aromatic hydrocarbons), and oxygen, a reaction occurs which may be very efficient. Acceptors such as dienes, dienoid heterocycles, and polycyclic aromatic compounds add oxygen to give endoperoxides (I in Fig. 1); olefins react to give hydroperoxides (II) (*7, 12*). The reactions are analogous to the Diels-Alder reaction, and the Alder "ene" reaction (*13*), respectively. The reactions are very smooth, and preparatively useful because of their high yields and specificity; the double bond of olefins always shifts to the allylic position. The following observations are relevant to the mechanism (*7, 14*). (i) The reaction proceeds by interaction of triplet sensitizer with oxygen (acceptors do not quench the triplet, in general). (ii) A new, kinetically distinguishable intermediate (X) is formed *quantitatively* from dye triplet at O_2 concentrations above $10^{-5}M$; sensitizer is regenerated, and can undergo hundreds of cycles. This reaction competes with and completely overshadows electron transfer to oxygen. (iii) The intermediate (X) either reacts ($k_A{}^X$) with acceptor (A) to give the product (AO_2) or decays ($k_d{}^X$); good acceptors trap it quantitatively even at low concentrations. The intermediate is electrophilic and very selective. It fails to react at all with most compounds (for example, alcohols); in this case the only observable effect is quenching of the triplet sensitizer. The reaction scheme is summarized below.

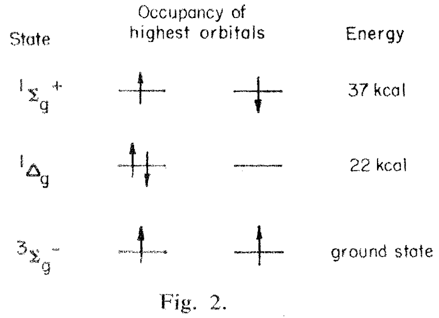


Fig. 2.

$$\begin{tikzcd} & & AO_2 \\ {}^3\text{Sens} \arrow[r, "O_2", "k_{O_2}"'] & X \arrow[ur, "A(k_A{}^X)"] \arrow[dr, "k_d{}^X"'] & \\ & & O_2 \end{tikzcd}$$

Two possibilities for X are consistent with the facts noted above: (i) excited oxygen, formed by energy transfer from dye to oxygen (*15, 16*)

$$^3\text{Sens} + O_2 \rightarrow \text{Sens} + O_2{}^*$$

or (ii) a sensitizer-oxygen complex ($\text{Sens-}O_2$) (*14, 17*)

$$^3\text{Sens} + O_2 \rightarrow \text{Sens} - O_2$$

It is experimentally very difficult to distinguish between these two mechanisms (which are kinetically equivalent); until recently, most workers preferred the sensitizer-oxygen complex. The discovery that excited-singlet molecular oxygen is produced in high yield in the chemiluminescent reaction between NaOCl and H_2O_2 (*18*) opened a new route to investigation.

$$\text{NaOCl} + H_2O_2 \rightarrow \text{NaCl} + H_2O_2 + {}^1O_2$$

Reactions of singlet oxygen. Oxygen has two metastable singlets, with spectroscopic symmetry notations $^1\Sigma_g{}^+$ (37 kilocalories) and $^1\Delta_g$ (22 kilocalories). The $^1\Delta_g$ state is long-lived and survives at least 10^8 collisions with methanol in the vapor phase, whereas the $^1\Sigma_g{}^+$ state survives no more than ten collisions under the same conditions (*19*). The $^1\Delta_g$ state is the principal product of the OCl^-/H_2O_2 reaction (*18*). The states of the oxygen molecule and their electronic configurations are shown in Fig. 2.

Singlet oxygen, produced in the hypochlorite-H_2O_2 reaction (*20, 21*) by electrodeless discharge (*22*) or in any of several other ways (*23–25*), reacts with dienes and olefins to give products identical to those of the photooxygenation.

In order to compare the reactions of chemically produced singlet oxygen with those of the reactive species in the photooxygenation, we have compared product distributions and stereoselectivity, relative reactivities of acceptors, and the ratio of the decay rate ($k_d{}^X$) to the reaction rate ($k_A{}^X$) of the intermediate for the two reactions.

With more than 30 different olefins which yield more than one product, the product distributions in the two reactions are identical, within the limits of experimental error of the analyses (*26, 27*). There are thus no detectable differences in stereoselectivity, a result which might have been expected were a bulky sensitizer complexed with the reactive intermediate at the transition state. The kinetic behavior of the two intermediates is also indistinguishable (*28*). The relative reactivities of a large number of different acceptors toward the intermediates in the two reactions were determined by competition experiments, and found to be the same, within the limits of experimental error. The ratio $(k_d{}^X)/(k_A{}^X)$ for the intermediate was determined by measuring product yield as a function of acceptor concentration, and was also found to be the same for the photochemical and nonphotochemical reactions. Any complexing of oxygen with sensitizer would be expected to alter both the rate of reaction and the rate of decay of the intermediate. The evidence thus favors the intermediacy of free singlet oxygen in the photooxygenation; experiments showing that the intermediate is volatile also support this conclusion (*15, 29, 30*). Arguments based on the lifetime of the intermediate ($\geqslant 10^{-6}$ second) and on the type of chemistry observed strongly suggest (*21*) that the oxygen is in the $^1\Delta_g$ state, rather than the $^1\Sigma_g{}^+$ state or a vibrationally excited ground state (*29*). A cautionary note should be added, however: the triphenylphosphite-ozone complex $[(C_6H_5O)_3PO_3]$ has been described as a source of singlet oxygen and is effective in carrying out oxygenations of the type described above (*25*). However, it has recently been reported that this complex reacts with acceptors at temperatures far below the point at which it gives off oxygen (*31*). The complex also appears to give relative reaction rates with acceptors which differ from those of the photochemical intermediate [in contrast to our results with the $H_2O_2\text{-}OCl^-$ reagent (*27, 28*)]. It seems likely that the triphenylphosphite-ozone complex acts as an oxygen transfer agent, and there may be various degrees of "freedom" of the singlet oxygen in certain cases.

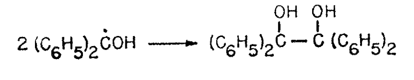
Possible involvement of $^1\Sigma_g{}^+ O_2$ in a photooxidation has recently been suggested (*32*). On theoretical grounds, Kearns had suggested that sensitizers of triplet energy (E_T) > 37 kilocalories (the energy of $^1\Sigma_g{}^+ O_2$) should produce $^1\Sigma_g{}^+$ oxygen as the primary product of energy transfer, whereas sensitizers with $E_T < 37$ kilocalories should produce only $^1\Delta_gO_2$ (*33*). Kearns *et al.* recently showed that an otherwise-difficult-to-explain sensitizer dependence of the photooxidation products from Δ^4-cholestene-3-β-ol could be rationalized on the basis of different reactions of $^1\Sigma_g{}^+$ and $^1\Delta_gO_2$ (*32*). However, the reported dependence of product on acceptor concentration (*34*) is not consistent with *any* simple kinetic scheme involving only $^1\Sigma_g{}^+$ and $^1\Delta_gO_2$ (*35*); further experimentation is necessary for a clarification of these phenomena.

Further complications are introduced by the fact that singlet oxygen can also be produced (probably in very low yield) from $O_2^-\cdot$ or $HO_2\cdot$) (*36*) and could, therefore, in principle, also be produced via the electron transfer path or by some of the mechanisms discussed in the next section; this path is probably of negligible importance, however. Koizumi has recently interpreted some of his data in terms of a short-lived complex, Sens+ . . . O_2^- (*37*) [this is one formulation for the sensitizer-oxygen complex (*38*)]; however, it is clear that Koizumi's complex cannot be intermediate in the photooxygenation reaction because it is formed in too low yield ($< 2 \times 10^{-3}$ of deactivating collisions) (*9*).

Sensitizer-Substrate Interaction

A bewildering variety of reactions is possible when either the oxygen pressure becomes low enough or enough of a sufficiently reactive substrate is present to make reaction between triplet sensitizer and substrate compete with oxygen quenching. The major modes of reaction are hydrogen-atom, electron, or energy transfer. Several very complex sequences of reactions have been shown to occur by these mechanisms in apparently simple systems. I will distinguish between primary reactions and the subsequent reactions of primary species.

Primary reactions. A very well established reaction between triplet sensitizer and substrate leads to oxidation of the substrate and reduction of the sensitizer in the primary step; both hydrogen-atom (*39*) and electron transfer reactions (*40*) have been shown to occur. Alcohols act as hydrogen donors for excited ketones and quinones and [much less efficiently relative to reaction with oxygen (*7*)] for dyes. Phenols and some amines are much more efficient reductants than alcohols, and are capable of reacting with excited dyes at rates competitive with that of oxygen (*41–43*). The consequences of hydrogen-atom and electron transfer are similar (the processes are often difficult to distinguish), and the primary products are radicals or radical ions.



$$2\,(C_6H_5)_2\dot{C}OH \longrightarrow (C_6H_5)_2\overset{OH}{C}-\overset{OH}{C}(C_6H_5)_2$$

III IV

Fig. 3.

Electron transfer (*e*-transfer) *from* sensitizer *to* an oxidizing substrate is also known (*43, 44*), although it appears to be less common. Metal ions such as Fe^{3+} are suitable oxidants; electron transfer to oxygen is mentioned above. Some electron-rich excited molecules eject electrons directly into solution, in a special case of this reaction (*45*). The various primary redox processes which occur on sensitizer-substrate interaction are shown below:

$$^3\text{Sens} + \text{SH} \xrightarrow[\text{H-atom transfer}]{} \text{Sens H}\cdot + \text{S}\cdot$$

$$^3\text{Sens} + \text{S} \xrightarrow[e\text{-transfer}]{} \text{Sens}^-\cdot + \text{S}^+\cdot$$

$$^3\text{Sens} + \text{S} \xrightarrow[e\text{-transfer}]{} \text{Sens}^+\cdot + \text{S}^-\cdot$$

$$\text{Sens}^* \xrightarrow[e\text{-ejection}]{} \text{Sens}^+\cdot + e^-\cdot(\text{solv.})$$

A further complication which can occur is that excited dyes can transfer electrons to ground-state dyes (Koizumi "D-D" mechanism) (*9, 10, 46, 47*). The rate constant ($k_{D\text{-}D}$) for this process is only slightly less than k_{O_2} for some dyes, so this reaction can become important at low O_2 or high dye concentration.

$$^3\text{Sens} + \text{Sens} \xrightarrow[k_{D\text{-}D}]{} \text{Sens}_{\text{ox}} + \text{Sens}_{\text{red}}$$

Reactions of primary species. The complexity of the sensitizer-substrate path is largely a result of the variety of secondary reactions open to the initially formed redox pair. Many of these processes can be detected only by means of sensitive techniques such as flash spectroscopy and electron spin resonance, which allow the observation of short-lived species.

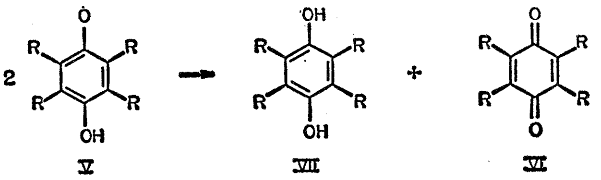
Electron and hydrogen-atom transfer reactions are often followed by a rapid reverse reaction regenerating the sensitizer in its ground state, so that the only observable effect is quenching of the excited sensitizer. [In some cases this process may produce excited species and lead to such phenomena as delayed fluorescence (*48*).] An example of this type of reaction is the quenching of eosin triplet by *p*-phenylenediamine (PPDA) (*44*).

$$^3\text{Eosin} + \text{PPDA} \longrightarrow \text{Eosin}_{\text{red}} + \text{PPDA}_{\text{ox}} \longrightarrow \text{Eosin} + \text{PDA}$$

A second reaction of importance is combination of radical species produced in electron or hydrogen-atom transfer reactions. For example, in the absence of air, hydroxybenzhydryl radicals (III in Fig. 3) formed by hydrogen abstraction by benzophenone dimerize, forming benzpinacol (IV) (*49*).

Instead of combining, intermediate radicals may disproportionate. For example, semiquinone radicals (V in Fig. 4) (or radical anions) yield quinone (VI) and hydroquinone (VII) (*50*). [This process is very common with dyes; equal amounts of leuco dye and starting dye are produced (*51*).]

Intermediate radicals can react rapidly with oxygen to give peroxy radicals which, in turn, abstract hydrogen to yield hydroperoxides (a process not to be confused with the formation of allylic hydroperoxides by the "singlet oxygen" mechanism). Depending on the source of the hydrogen, new radicals may or may not be produced, and may result in conventional radical chain autoxidation (*52*). An example of a reaction which does not go by way of a chain is the reaction with oxygen of hydroxyisopropyl radicals (VIII in Fig. 5), formed by hydrogen abstraction from isopropyl alcohol by triplet benzophenone, to give peroxyradicals (IX); these are reduced by the semireduced benzophenone radicals to give the final product hydroxyhydroperoxide (X) and regenerate benzophenone (*53*).



V VII VI

Fig. 4.

Electron or hydrogen-atom transfer reactions of the primarily produced radical species are often very important. For example, oxygen reacts rapidly with semireduced dye intermediates (*10, 51*) such as that from eosin, to give the regenerated dye and $O_2^{-}\cdot$. [The principal reaction of $O_2^{-}\cdot$ in solution seems to be disproportionation (*54*).]

$$\text{Eosin}_{\text{red}} + O_2 \longrightarrow \text{Eosin} + O_2^{-}\cdot$$
$$2\,O_2^{-}\cdot \xrightarrow[2H^{+}]{} O_2 + H_2O_2$$

In a similar reaction in the absence of oxygen, hydroxyisopropyl radicals (VIII in Fig. 6) produced from isopropyl alcohol by hydrogen abstraction are further oxidized by benzophenone to form acetone (*49*).

Similar reactions of primary intermediates have been shown to be important in several cases where apparently simple overall reactions occur; otherwise invisible intermediates have been observed by flash spectroscopy (*9, 10, 37, 46, 47*). An example is the anaerobic oxidation of allylthiourea (ATU) by eosin at high concentrations, which proceeds via the D-D mechanism. [The direct process, with the same overall reaction, occurs at lower dye concentrations (*9*)].

$$^{3}\text{Eosin} \longrightarrow \text{eosin}_{\text{ox}} + \text{eosin}_{\text{red}}$$
$$\text{Eosin}_{\text{ox}} + \text{ATU} \longrightarrow \text{eosin} + \text{ATU}_{\text{ox}}$$
$$\text{Overall: } {}^{3}\text{eosin} + \text{ATU} \longrightarrow \text{eosin}_{\text{red}} + \text{ATU}_{\text{ox}}$$

A second example of this type of indirect mechanism occurs in the reaction between methylene blue (MB), ferric ion, and ethylenediaminetetraacetic acid (EDTA) (*55*).

$$^{3}\text{MB} + \text{EDTA} \longrightarrow \text{MB}_{\text{red}} + \text{EDTA}_{\text{ox}}$$
$$\text{MB}_{\text{red}} + \text{Fe}^{3+} \longrightarrow \text{MB} + \text{Fe}^{2+}$$
$$\text{Overall: } \text{EDTA} + \text{Fe}^{3+} \xrightarrow[h\nu/\text{Sens}]{} \text{EDTA}_{\text{ox}} + \text{Fe}^{2+}$$

It has often been proposed that dye-sensitized photooxidations proceed via energy transfer from triplet sensitizer to acceptor, followed by oxidation of the excited acceptor (*56*). A requirement for energy transfer is that the acceptor must have a triplet energy lower than that of the sensitizer; triplet levels of sensitizing dyes are very low (on the order of 40 kilocalories), so this condition will rarely be satisfied (*57*). One case where energy transfer apparently occurs is in the sensitized decomposition of the polyene fungicide pimaricin (*58*); O_2 is not required, so this is not a reaction of the general "photodynamic" type and, in fact, is probably not an oxidation. Triplets are usually quenched by oxygen (with energy transfer), rather than reacting with it.

$$^{3}\text{Sens} + \text{A} \longrightarrow \text{Sens} + {}^{3}\text{A}$$
$$^{3}\text{A} \xrightarrow[\text{oxidant}]{} \text{A}_{\text{ox}}$$

In addition, mechanisms involving excitation of a ground-state complex (Sens. . .A) (*59–61*) or formation of an excited-state complex (Sens. . .A)* of sensitizer and acceptor (*62*) have been proposed.

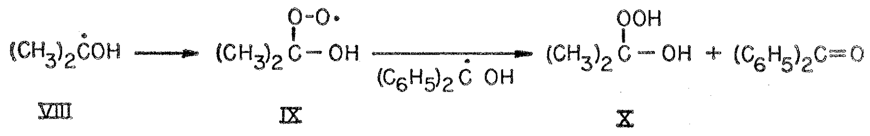
$$\text{Sens} + \text{A} \rightleftarrows \text{Sens} \ldots \text{A} \xrightarrow[h\nu]{}$$
$$(\text{Sens} \ldots \text{A})^{*} \xrightarrow[\text{oxidant}]{} \text{products}$$
$$^{3}\text{Sens} + \text{A} \longrightarrow (\text{Sens} \ldots \text{A})^{*} \xrightarrow[\text{oxidant}]{} \text{products}$$

Complexing probably is important in macromolecular systems, partly because the yield and lifetime of triplet are affected (*60, 61*). Excited-state complexing mechanisms were originally proposed for photooxidation of the aromatic hydrocarbon rubrene (*62*); however, this reaction goes by the singlet oxygen mechanism (*7*). Both ground- and excited-state complexing mechanisms can lead to the same consequences as other sensitizer-substrate reactions. With furocumarins, permanent covalent bonding of sensitizer to substrate occurs; the reaction occurs in the absence of oxygen and is, therefore, not of the ordinary photodynamic type (*63*).

Summary of Mechanisms

The scheme of Figs. 7 and 8 summarizes the most important of the mechanisms of photosensitized oxidation which have been discussed. Substrate (S) may be solvent (for example, alcohols) or an added solute; acceptors (A) are compounds which react in the photooxygenation (singlet oxygen type) reaction; under certain conditions they can also serve as substrates for the direct reaction with sensitizer (to give radical-type products).

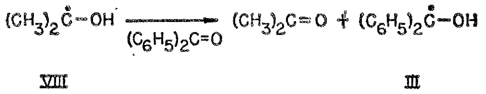
It is obvious that the actual mechanism or combination of mechanisms



$$(CH_3)_2\dot{C}OH \longrightarrow (CH_3)_2\overset{\text{O-O}\cdot}{C}-OH \xrightarrow[(C_6H_5)_2\dot{C}OH]{} (CH_3)_2\overset{\text{OOH}}{C}-OH + (C_6H_5)_2C{=}O$$

VIII IX X

Fig. 5.



$$(CH_3)_2\dot{C}{-}OH \xrightarrow[(C_6H_5)_2C=O]{} (CH_3)_2C{=}O + (C_6H_5)_2\dot{C}{-}OH$$

VIII III

Fig. 6.

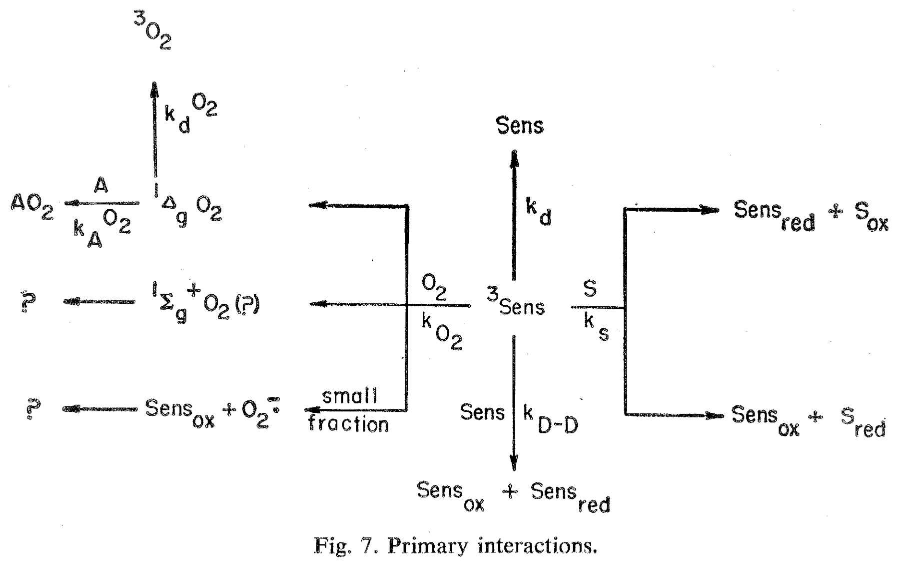


Fig. 7. Primary interactions.

which occurs in a given photooxidation system is a complex function of conditions. The large value of k_{O_2} makes reaction with O_2 predominate in oxygenated systems of dyes, except at very high concentrations of dye or powerful reductants, or at very low oxygen concentrations; however, in the absence of a suitable acceptor for $^1O_2$, the singlet oxygen part of this path leads only to quenching, and low-efficiency processes via other paths may become visible. In biological systems, binding of sensitizers to macromolecules (*60*, *61*) would be expected to favor sensitizer-substrate interactions even at high O_2 concentrations; dye aggregation should favor the D-D mechanism. In addition, the reactions with oxidizable substrates of H_2O_2 formed in various processes must be considered, and concurrent nonphotochemical autoxidation may occur, particularly if peroxides which act as initiators are formed in the photochemical reaction. A considerable part of the confusion in the literature has resulted from attempts to explain all sensitized oxidation phenomena by one mechanism.

In light of the above scheme, it is worth discussing the reported oxidations of some biologically important substrates under photodynamic conditions (dye-sensitized, aerobic). Macromolecular systems are not discussed here because the importance of substrate-dye binding is difficult to evaluate. I attempt to distinguish only between "singlet oxygen" (sensitizer-O_2 path) and "radical" (sensitizer-substrate path) chemistry.

Biological Substrates

Enamines. As a model for heterocyclic compounds of biological interest, the photooxidation of enamines has been studied (*64*). Even in cases in which no allylic hydroperoxide can be formed, cleavage of the C-C double bond occurs smoothly. A sensitizer-substrate mechanism has not been ruled out, but a singlet oxygen mechanism appears more likely. An oxygenated intermediate is formed which is stable at low temperatures. The structure of the intermediate is not certain, but it may be a dimeric peroxide of structure XI (Fig. 9). A simple monomeric dioxetane (XII) or zwitterion (XIII) is not consistent with the nuclear magnetic resonance spectrum of the intermediate; XII or XIII may, however, be intermediate in the formation of XI.

Histidine. The oxidative destruction of histidine is the major cause of deactivation of many enzymes (*2*). The photooxidation is efficient, and only the neutral form reacts (*65–67*). The products have not been established, but the point of attack is the imidazole ring, which is cleaved (*68*, *69*). From the reported kinetics, the mechanism of the proflavin- and methylene-blue-sensitized oxidation appears to be the same as that of furfurol (*65*, *66*, *70*, *71*); furans (*20*, *21*), oxazoles (*24*, *72*), and imidazoles (*73*, *74*) are excellent acceptors for chemically generated singlet oxygen. Triphenyl imidazole (XIV in Fig. 10) gives the hydroperoxide (XV), which cleaves under mild conditions to give product XVI (*73*).

Tetraphenylimidazole cleaves in an exactly analogous fashion; however, in this case [as with the enamines (*64*)], a hydroperoxide cannot be an intermediate (*74*); the nature of the actual intermediate has not yet been established, but it may be analogous to that in the enamine case. The oxidation of histidine probably also proceeds by a singlet oxygen mechanism to give analogous products, although, at low oxygen pressures, a contribution from a sensitizer-substrate mechanism is probable (*71*). *N*-Benzoyl histidine gives aspartic acid in a reaction which can be rationalized in a similar manner (*74*).

Methionine. Methylene-blue- and riboflavin-sensitized oxygenation of methionine produces methionine sulfoxide (*65*, *69*, *75*, *76*). Since the points of attack of aerobic oxidation (the sulfur) and anaerobic oxidation (probably hydrogen on the carbon in the position α to the amino group) (*75*) are different, the evidence is against a sensitizer-substrate mechanism for the aerobic oxidation. The kinetics appear consistent with a singlet oxygen mechanism (*65*, *70*). Photosensitized oxidation of simple dialkyl sulfides similarly produces sulfoxides in a very efficient reaction (*7*, *77*). Preliminary experiments support the interpretation that olefins and sul-

$$\mathrm{Sens_{red}} + \mathrm{S_{ox}}\ (\text{or } \mathrm{Sens_{ox}}) \longrightarrow \mathrm{Sens} + \mathrm{S}\ (\text{or Sens})$$

$$2\,\mathrm{Sens_{red}}\ (\text{or } \mathrm{S_{ox}}) \longrightarrow \text{Dimeric products}$$

$$2\,\mathrm{Sens_{red}}\ (\text{or } \mathrm{S_{ox}}) \longrightarrow \text{Disproportionation}$$

$$\mathrm{Sens_{red}}\ (\text{or } \mathrm{S_{ox}}) \xrightarrow[O_2]{} \text{peroxyradicals} \longrightarrow \text{hydroperoxides}$$

$$\mathrm{Sens_{red}}\ (\text{or } \mathrm{S_{red}^{\cdot}}) + \text{oxidant} \longrightarrow \mathrm{Sens}\ (\text{or S}) + \text{oxidant}_{red}$$

$$\mathrm{S_{ox}}\ (\text{or } \mathrm{Sens_{ox}}) + \text{reductant} \longrightarrow \mathrm{S}\ (\text{or Sens}) + \text{reductant}_{ox}$$

$$2\,O_2^{\cdot -} \xrightarrow[2H^+]{} O_2 + H_2O_2 \ \text{(other reactions of } O_2^{\cdot -} \text{ may possibly occur.)}$$

Fig. 8. Reactions of primary products.

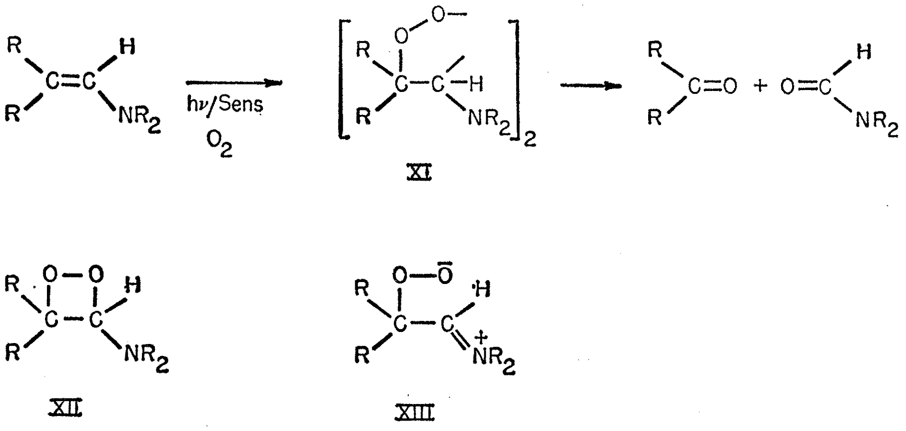


Fig. 9.

fides compete for a common intermediate (*78*) and thus both proceed via the singlet oxygen path. The reaction can be formulated as proceeding via an unstable persulfoxide (XVII in Fig. 11), which oxidizes a second mole of sulfide (two moles of sulfide are consumed per mole of oxygen) (*77*). Further oxidation to the sulfone is much slower (*79*).

Tryptophan. The kinetics of the methylene-blue-sensitized oxidation of tryptophan are not consistent with a pure singlet oxygen mechanism (*65, 70*). Grossweiner and Zwicker reported observing tryptophyl radicals from eosin and ovalbumin by electron spin resonance even in the presence of air (*80*). The major product is *N*-formylkynurenine (XVIII in Fig. 12) (*81*), which is closely analogous to the products of cleavage of enamines and of imidazoles discussed above. However, indoles are very sensitive to cleavage of the pyrrole ring in nonphotochemical oxidations (*82*). The data are insufficient for a definite statement about mechanism at this time.

As mentioned above, phenols are much more powerful reductants for excited dyes than alcohols are. For example, with eosin and phenol, $k_{O_2}/k_S = 0.2$ (*41*). Thus, in moderate concentration, phenols can be oxidized by direct interaction with excited dyes even in the presence of oxygen. The products from 2,6-di-*t*-butyl phenol (XIX in Fig. 13) and other hindered phenols have been characterized, and are in accord with a mechanism involving phenoxy radicals (*83*).

The kinetics of methylene-blue-sensitized tyrosine oxidation are not consistent with a pure singlet oxygen mechanism (*65, 70*). Strong evidence has been presented for a dye-substrate interaction in aerobic eosin-sensitized tyrosine oxidations (*41, 80*). All evidence thus favors a sensitizer-substrate reaction; since the reaction proceeds best via the anion (*65, 67*), an electron transfer mechanism seems attractive. The aromatic ring is believed to be cleaved (*69*).

Other amino acids and amines. With the exception of cysteine, which is oxidized in the dark as well (*84*), most other amino acids are relatively inert to photosensitized oxidation. Cystine is slowly oxidized, apparently via the sulfoxide (*69*).

Secondary and tertiary amines are oxidized; the products are poorly characterized. Reports of both *N*-oxide (*85*)

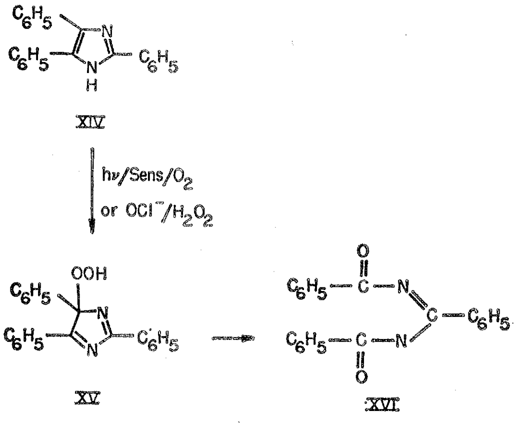


Fig. 10.

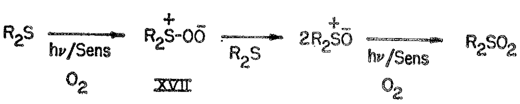


Fig. 11.

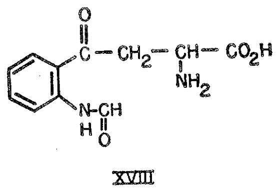


Fig. 12.

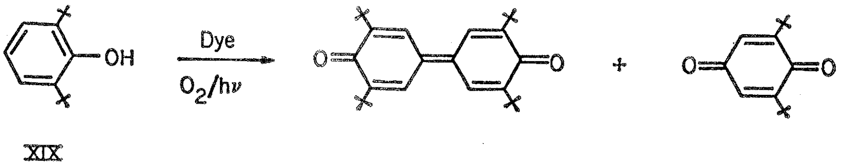


Fig. 13.

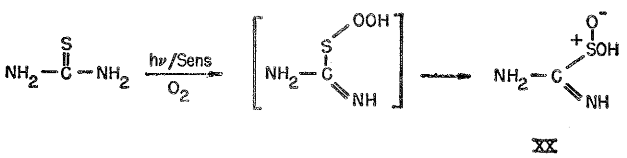


Fig. 14.

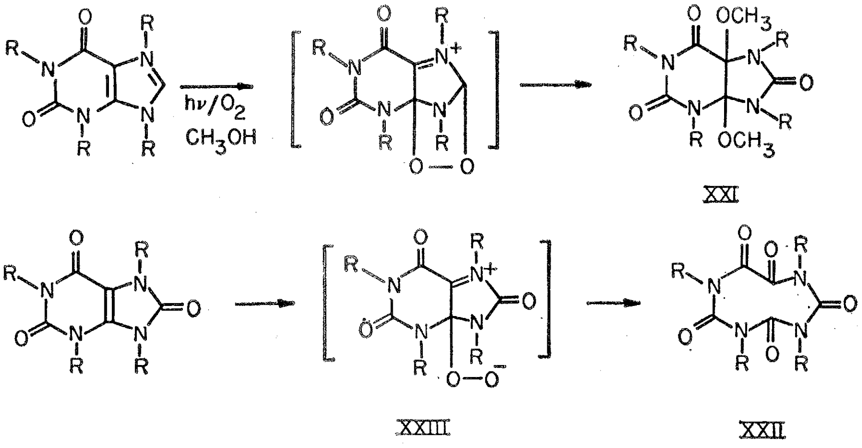


Fig. 15.

and α-hydroperoxide (*7, 86*) formation have been published. Both reports may be correct, as the products are not isolated in high yield and amines react rapidly with peroxides to give *N*-oxides. Anaerobically, amines are oxidized by benzophenone to form imines by an electron or hydrogen transfer mechanism (*87*). Very little is known about any of these reactions.

Allylthiourea has been extensively studied as a model photooxidation substrate. With dyes, $k_{\mathrm{O}_2}/k_{\mathrm{S}}$ is such that the aerobic oxidation at low substrate and dye concentrations proceeds via a sensitizer-oxygen path, thus probably via a singlet oxygen mechanism (*9, 47*). Thiourea oxidation produces a sulfinic acid (XX in Fig. 14), the expected product of singlet oxygen attack (*88*). The corresponding product is probably formed from allylthiourea, but is unstable under the reaction conditions (*88*).

Guanine derivatives. The chemistry of guanine photooxidation is only partly understood, and very little mechanistic work has been done. The primary isolable products are often those of extensive degradation (*89, 90*); they can be explained in terms of a singlet oxygen mechanism, as can the products of oxidation of other purines (*90, 91*).

Evidence for cleavage of the type observed with enamines and histidine has recently been reported by Matsuura (*92*). Products of type XXI or XXII (Fig. 15), depending on the substituent, are among those formed on photooxidation of purines.

Product XXI is exactly analogous to products found on photooxidation of furans in alcohols (*93*) or of some imidazole derivatives (*74*). Matsuura suggests a zwitterion intermediate (XXIII) in the formation of product XXII (*92*). Such a zwitterion could also account for the formation of dimeric peroxide intermediates in enamine oxidation, and for the imidazole cleavage reactions described above.

Effect of Carotenoids

Chlorophyll is an excellent photosensitizer for all types of photooxidation (*5, 7, 14*). Photosynthetic organisms, however, are apparently protected by carotenoids against the lethal effects of their own chlorophyll and of exogenous sensitizers; mutants lacking carotenoids are rapidly killed in the presence of light and oxygen (*94, 95*). The protective effect of carotenoids is well established but not well understood; it has been suggested that this protective action is a principal function (*94*).

It is well known that carotenoids quench triplet molecules efficiently (*96*). We have recently found that β-carotene in solution also is an extremely efficient quencher for singlet oxygen (*97*). For example, $10^{-4}M$ β-carotene inhibits 95 percent of the methylene-blue-sensitized oxidation of $0.1M$ 2-methyl-2-pentene. It can be shown kinetically that, under these conditions, triplet methylene blue is not being quenched appreciably (the oxygen concentration is 100 times that of β-carotene). Although the triplet energy of β-carotene is apparently not known, it may well be low enough so that $^1\Delta_g$-excited oxygen can transfer energy to it, producing triplet carotene and ground state oxygen. (However, other mechanisms for the quenching are possible.) The fate of the triplet carotene is not established, but most of it must return to the ground state, since one molecule of β-carotene quenches at least 100 molecules of singlet oxygen. β-Carotene is equally efficient in inhibiting oxygenation of 2-methyl-2-pentene by chemically produced singlet oxygen. These results are preliminary and subject to refinement, but they must have some relevance to the protective action of carotenoids. In addition, if triplet carotene is produced in the quenching process and can perform a role in the photosynthetic cycle, a double function would be served: the toxic singlet oxygen would be removed, and the energy it had received from the chlorophyll, which would otherwise be lost, would be recovered as carotene excitation:

$$^3\text{Chlorophyll} + {}^3\mathrm{O}_2 \longrightarrow \text{chlorophyll} + {}^1\mathrm{O}_2$$

$$^1\mathrm{O}_2 + \beta\text{-carotene} \rightarrow {}^3\mathrm{O}_2 + {}^3\beta\text{-carotene (?)}$$

These interpretations are highly speculative but suggest further experiments.

Summary. The many established mechanisms of photosensitized oxidation are broadly grouped into two classes—those involving primary interaction of excited sensitizer with oxygen or those involving its primary interaction with another molecule; the relative importance of the two paths depends on reaction conditions. Interaction with oxygen produces singlet oxygen, which is the reactive species in this type of oxidation. Mechanisms can be tentatively assigned to the photooxidations of some biological molecules; some photooxidations apparently proceed via a singlet oxygen mechanism, but others do not. New results suggest that part of the protective action of carotenes in biological systems may possibly derive from their ability to quench singlet oxygen.

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Computer-Managed Instruction

The use of computers as a teacher's aid may entirely revolutionize the field of education.

Harvey J. Brudner

During the 20 years since the first commercial electronic data processing machines went into production, computers have become smaller, less expensive per unit computation, significantly faster, and have been given vast memories. Over 50,000 have been installed in the United States; however, even though almost 8 percent of our gross national product is in some way related to education and training, a much smaller percentage of the nation's computer power is involved with education. Computers are used most for record keeping, scheduling, payroll, and other administrative tasks. Part of the reason for the lag relates to how much information can be economically stored in and retrieved from a computer.

The state of the art of computer-assisted instruction has been comprehensively reviewed by Atkinson and Wilson (*1*). An economic assessment of computer-assisted instruction (*2*), based on over 2 years of analysis, indicates that annual costs are now about $400 per student for the drill and practice mode, and about $1000 per student for the tutorial mode. Furthermore, if computer-assisted instruction were extended throughout the nation's public school systems, the annual costs could range from $9 billion to $24 billion.

With the total annual public school expenditure in the United States at about $30 billion, or some $600 per student, other approaches should be

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