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The Chemical Properties of Singlet Molecular Oxygen and Its Importance in Biological Systems

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Typical chemical reactions of singlet molecular oxygen with organic substances as well as its disruptive influence on biological systems (the photodynamic effect) are described. The involvement of singlet oxygen in carcinogenesis is demonstrated. A new (luminescence) method of detecting singlet oxygen in solution is discussed. The role of singlet molecular oxygen as an oxidant in polluted city air is noted.
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I. CHEMICAL REACTIONS OF SINGLET OXYGEN WITH ORGANIC SUBSTRATES

The oxygen molecule has three possible lowest electronic states which differ in the population of their highest-lying molecular orbitals (Fig.1). The first state ($^3\Sigma$) has the lowest energy, since the two electrons in the highest orbitals are located in different regions of space and consequently their mutual repulsion is minimal. The second state ($^1\Delta$) has a higher energy, since greater interelectron repulsion corresponds to two electrons in the same orbital $\pi^*(2p_y)$. The energy of the third state ($^1\Sigma$) is higher still, since its attainment requires the expenditure of a fairly large amount of energy in order to reverse the spin of the electron in one of the π^* orbitals.

The first state is the ground triplet state and the other two are excited singled states. The singlet states $^1\Delta$ and $^1\Sigma$ lie 22.5 and 37.5 kcal mol^{-1} above the ground state[1]. Singlet molecular oxygen is in general designated by $^1O_2$ in contrast to ordinary oxygen which is designated by $^3O_2$ (or simply by O_2). Fig.1 shows that oxygen in the ground state or "triplet" oxygen has two unpaired electrons; it is a stable biradical of the type $^{\cdot}O-O^{\cdot}$ and its chemical reactions are of the free radical type[2]. As an example, one may quote the radical chain autoxidation of isopropyl alcohol:

$$HOC(Me)_2H \xrightarrow[-\dot{O}_2H]{^3O_2} HO\dot{C}(Me)_2 \xrightarrow{^3O_2} \longrightarrow HOC(Me)_2O-O^{\cdot} \xrightarrow[-HO\dot{C}(Me)_2]{HC(Me)_2OH} HOC(Me)_2OOH.$$

It was initially postulated[3-6] that the $^1\Sigma$ state must exhibit a similar reactivity. However, it has been established[7] that the $^1\Sigma$ state relaxes in solution to the $^1\Delta$ state over a period of approximately 10^{-10} s and the authors[8,9] therefore concluded that the reactions of $O_2(^1\Sigma)$ cannot be detected. Additional experimental data, confirming this conclusion, have

been obtained[10]; the author of the above study, who used a wide range of photosensitisers to investigate the reactions of singlet oxygen, established that the proportions of the reaction products do not change when the energy of the triplet state of the sensitiser is below the energy required for the formation of $O_2(^1\Sigma)$. Furthermore, according to the theory $O_2(^1\Sigma)$ cannot undergo concerted addition reactions[11], whereas the reactions of singlet oxygen are concerted[12-14]. We shall therefore consider below only the reactions of the $O_2(^1\Delta)$ form.

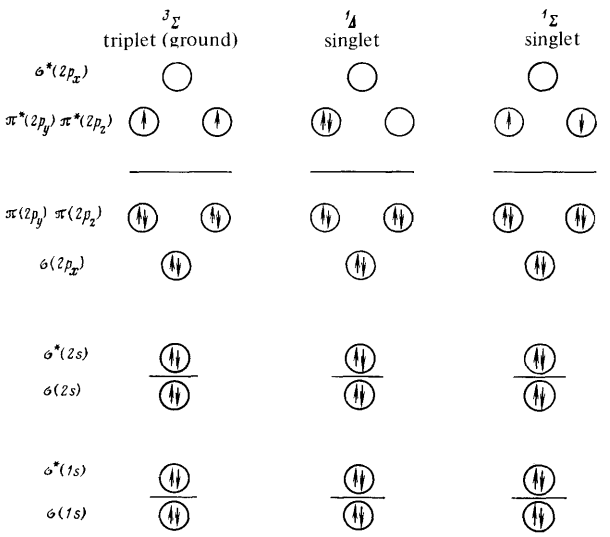
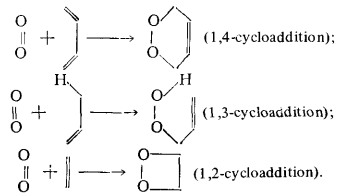


Figure 1. Possible electronic states in the oxygen molecule.

Singlet oxygen in the $^1\Delta$ state is extremely reactive, since, apart from the fact that it has an excess of energy, its lifetime is anomalously long (2700 s in the gas phase at a low pressure and approximately 10^{-5} s in solution).[15,16] The molecule of singlet oxygen in the $^1\Delta$ state can be represented by O=O (Fig.1), i.e. in its electronic structure it resembles ethylene and behaves in reactions as a dienophile[17-19].

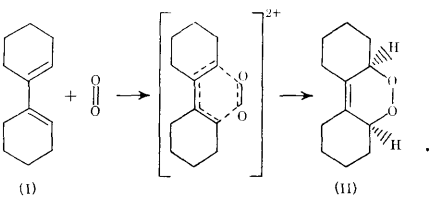
Thus the chemical properties of singlet oxygen in the $^1\Delta$ state are completely different from those of oxygen in the ground state. For example, the characteristic reactions of $O_2(^1\Delta)$ are 1,4-cycloaddition of the Diels–Alder type and 1,3- and 1,2-cycloaddition[11,20-28]:



These three types of reactions give rise to simple methods for the introduction of the peroxy-linkage into organic substrates and are important for the synthesis of new compounds. There is a multiplicity of examples of each type of cycloaddition, only some of which are presented below. More detailed data on the chemistry of singlet oxygen can be found in various reviews[10,11,17,21,22,28-31].

1. 1,4-Cycloaddition (the Endoperoxide Pathway)

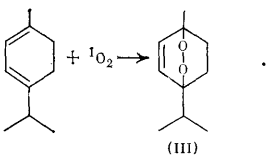
The concerted nature of the reaction involving cycloaddition to dienes has been observed in the addition of oxygen to 1,1'-bicyclohexenyl (I) in the *cis*-conformation. The reaction yields exclusively the *cis*-peroxide (II):[21]



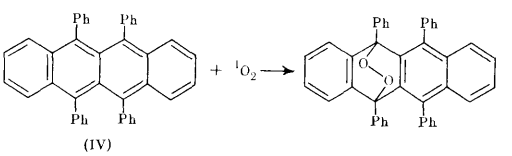
The addition of singlet oxygen proceeds via the formation of a six-membered cyclic transition state, as in the Diels–Alder reaction[10,17].

In the study of the effect of temperature on the rate of reaction of singlet oxygen with a series of dienes, it was found that the activation energy varies from zero for the more reactive dienes to approximately 6 kcal mol^{-1} for comparatively inert dienes[32]. The concerted nature of the reaction and the low activation energy for the addition of singlet oxygen to dienes agree with the theoretical results[11].

Numerous studies have been made on the reactions involving the addition of $^1O_2$ to dienes[21,27]. The *endo*-peroxide (III) (ascaridole) was isolated already in the first half of this century from chenopodium oil and was subsequently found to be formed in plant leaves in the course of the chlorophyll-sensitised oxidation of α-terpinene:[33,34]

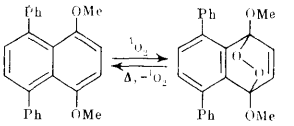


In the laboratory this reaction was discovered in connection with a fundamental study of the photochemical conversion of steroids into vitamin D,[35] where it was shown that ergosterol gives rise to an *endo*-peroxide on oxidation with a dye sensitiser. Extensive studies on the photochemical oxidation of steroid dienes were subsequently carried out[21] and it was observed that aromatic hydrocarbons, for example rubrene (IV), are converted into *endo*-peroxides under similar conditions:[36]



Later numerous studies were made on the photochemical oxidation of polycyclic aromatic and heteroaromatic compounds[21,27,37]. It was found that 9,10-di-substituted anthracenes and polycyclic aromatic compounds which are large molecules react with singlet oxygen almost quantitatively[21,27,37,38]. One should note that, in the photochemical oxidation of hydrocarbons of the acene series, there is no need to employ special sensitiser dyes, since acenes are themselves capable of acting as sensitisers; this also applies to rubrene.

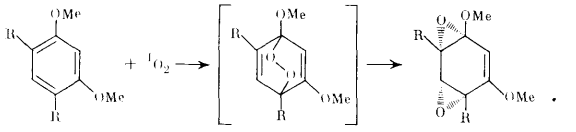
Activated derivatives of naphthalene and benzene also enter into the 1,4-cycloaddition reaction with singlet oxygen; for example, compound (V) can be converted into the *endo*-peroxide (VI):[39]



(V) (VI)

A series of di-, tri-, tetra-, hexa-, and octa-methylnaphthalenes have also been converted into *endo*-peroxides under the influence of $^1O_2$.[40,41]

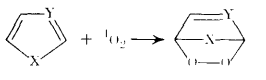
It has been shown that a derivative of resorcinol (VII) is converted into the *endo*-peroxide (VIII) on interaction with $^1O_2$, which results in the formation of the diepoxide (IX):[42]



(VII) (VIII) (IX)

R = t-Bu

A very large series of heterocyclic compounds, particularly five-membered compounds such as furans, thiophens, pyrroles, oxazoles, thiazoles, and imidazoles, undergo the 1,4-cycloaddition reaction with singlet oxygen[21,27]:



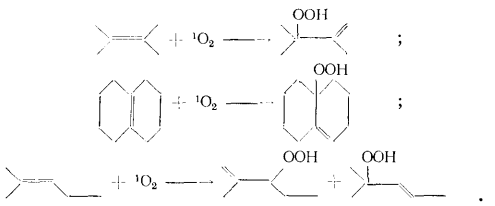
X = O, S, NH, NR; Y = CH, N.

endo-Peroxy-products are unstable and are converted into other compounds or are solvolysed.

2. 1,3-Cycloaddition (the Allylic Hydroperoxide Pathway)

In reactions of this type singlet oxygen adds to olefins, forming allyl hydroperoxides, as in the Alder reaction. The 1,3-cycloaddition mechanism has not so far been established exactly; nevertheless this process is of great synthetic importance, particularly for the series of terpenes, which are the main constituents of various essential oils isolated from flowers, fruits, leaves, and other parts of plants[28]. The reduction of allyl hydroperoxides to allyl alcohols can be achieved on an industrial scale in order to obtain synthetic perfumes.

Numerous studies have been made on 1,3-cycloaddition[10,15,17,22,30,43-49]. Some examples of reactions of this type are presented below:



The experimental observations concerning these reactions can be summarised as follows[11]:

(1) Oxygen is always introduced into the part of the molecule from which hydrogen is removed and the double bond always migrates[10,17,22,29,30,50].

(2) The reaction cannot be inhibited by adding radical trapping agents[50].

(3) For substituted olefins, the ratio of secondary and tertiary hydroperoxides is approximately 1 : 1.[10,17,22,29,30,51]

(4) Groups of substituents which shield the double bond prevent attack by singlet oxygen[52].

(5) There is no loss of optical activity in the oxidation of optically active olefins[50,53]. Furthermore, comparison of the photochemical oxidation reactions of *cis–trans* olefin pairs has shown that *cis–trans* isomerisation does not occur in the course of the reaction[51].

(6) The reactivity of olefins intensifies after the introduction of alkyl substituents (by a factor of approximately 10 per CH_3 group).[19]

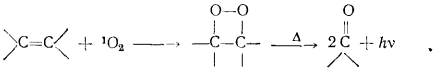
(7) For the majority of olefins, the nature of the solvent has very little influence on the proportions of the products or on the rate of reaction with singlet oxygen[10,17,22,29,30].

(8) There is a satisfactory correlation between the relative rates of reaction of olefins with peracids and with singlet oxygen[19].

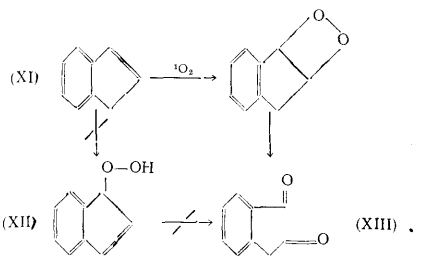
(9) There is a large difference between the rates of reaction of various substituted olefins with singlet oxygen and with free radicals. In the case of singlet oxygen the relative reactivity varies by four orders of magnitude depending on the structure of the olefin, while in the case of trifluoromethyl radicals the variation is only by a factor of two.[19]

3. 1,2-Cycloaddition (The 1,2-Dioxetan Pathway)

One of the most interesting achievements in the chemistry of singlet oxygen has been the discovery of a method for the 1,2-cycloaddition of singlet oxygen to olefins, which leads to the formation of "high energy" 1,2-dioxetans, which emit light and decompose into the corresponding carbonyl products[22]:



The first successful synthesis of a stable dioxetan was reported in 1968.[54,55] This was important, since it had been postulated earlier that carbonyl fragments, which frequently appear in photochemical oxidation reactions, arise as a result of secondary reactions of the unstable allyl hydroperoxides formed initially[17] and not as a result of the cleavage of the intermediate dioxetan. In the same year Foote and Lin[56] and also Huber[57] put forward the hypothesis that dioxetan is one of the three possible intermediates in the reaction of singlet oxygen with enamines. The role of dioxetan as an intermediate in the reactions of singlet oxygen with electron-rich olefins was finally established in a study[58], where it was shown that the dialdehyde (XIII), the only product of the sensitised photochemical oxidation of indene (XI), does not arise as a consequence of the rearrangement of the unstable allyl hydroperoxide (XII):[58]



(XI) (XII) (XIII)

There are many examples of rapid reactions of singlet oxygen with olefins despite the fact that the latter are unable to split off hydrogen atoms in allyl positions[59], which may indicate the formation of dioxetans.

Nitrogen-activated olefins such as enamines, bisacrylidylenes[60,61], indoles[62,63], and dipyrrylmethylenes[64] enter into the 1,2-cycloaddition reaction with $^1O_2$. Alkylthio-substituents are just as effective in activating the ethylene bond with respect to oxidation by singlet oxygen. For example, tetrathioethylenes, which are extremely stable in relation to the usual electrophiles and dienophiles, readily combine with $^1O_2$ to form dioxetans[65,66]. Even monothioethylenes undergo 1,2-cycloaddition[67] rather than attack on the sulphur atom by singlet oxygen[68,69].

II. BIOLOGICAL IMPORTANCE OF SINGLET OXYGEN

1. The Photodynamic Effect

Numerous studies have established[70–79] that virtually all types of biological systems, ranging from the simplest unicellular organisms to multicellular plants and animals, can be damaged by the simultaneous operation of the following three factors: light, oxygen, and a photosensitiser (it is usually a dye); this phenomenon was first discovered in 1900 and was called the photodynamic effect[71,72]. The photodynamic effect can lead to the damage of viruses, fungi, algae, and the simplest unicellular organisms, causing mutations, membrane defects, disturbance of metabolism, etc. (up to the lethal outcome). A number of reviews have been devoted to this effect[70,73–81]+.

The photodynamic effect is based on the photosensitised oxidation of the substrate during which the sensitiser molecule S absorbs a light quantum and passes to an excited singlet state ^1S:

$$S + h\nu \rightarrow {}^1S \quad .$$

From the ^1S state, the sensitiser can pass to an excited triplet state ^3S, which has a lower energy but is longer-lived:

$$^1S \rightarrow {}^3S \quad .$$

This state is in fact involved in the majority of photochemical and photophysical processes. Two main types of reactions of sensitisers in the triplet state have been discovered[17,30]. In reactions of type (I) the sensitiser interacts directly with the substrate, the process involving the abstraction of a hydrogen atom or the transfer of an electron, which leads to the oxidation of the substrate and the reduction of the sensitiser[30]. In reactions of type (II) the triplet sensitiser interacts with oxygen.

According to Kautsky's mechanism[82], which is generally accepted nowadays, the interaction of a sensitiser in the triplet state with oxygen in the ground state involves energy transfer from the sensitiser to oxygen, which results in the formation of singlet oxygen $^1O_2$:

$$^3S + {}^3O_2 \rightarrow S + {}^1O_2 \quad .$$

The singlet oxygen formed then attacks the substrate; in biological systems such substrates are, for example, certain aminoacid residues in proteins, the bases in nucleic acids, and the lipids in membranes. The rate constants for processes of type (I) are $\leq 10^7$ litre mol^{-1} s^{-1}, while those for processes of type (II) are greater by two orders of magnitude and amount to 1×10^9 litre mol^{-1} s^{-1}.[79] In this review only type (II) processes will be considered#. The range of the possible photosensitisers is extremely wide; it consists of dyes (Bengal Pink, Methylene Blue, acridines, etc.), natural pigments (chlorophyll, haematoporphyrin, flavins, etc.), and aromatic hydrocarbons (certain anthracenes, rubrene, etc.). The main condition governing the suitability of the compound as a sensitiser for the reactions considered is that the energy of its triplet state should be not less than 22.5 kcal mol^{-1}, i.e. the energy required for the transition of the oxygen molecule to the singlet state.

It is noteworthy that many medicinal substances such as antibiotics, phenothiazines, and sulphonamides are photosensitisers and can therefore give rise to photodynamic damage in the human organism. For example, it has been observed[84] that the antiobiotics tetracycline, oxytetracycline, and griseofulvin cause a pathological sensitivity of human skin to light.

(a) Protection of organisms against the destructive influence of the photodynamic effect. At the end of the 1960's, it was established that carotenoids can protect organisms against the destructive influence of the photodynamic effect[85]. It was shown on model systems[86] that the oxidation of the substrate by singlet oxygen is effectively inhibited by β-carotene.

The hypothesis that carotenoids can protect photosynthesising systems against the photodynamic effect (when they are sensitised by their own chlorophyll) was put forward as early as 1959 on the basis of experiments with carotenoid-free mutants of photosynthesising bacteria[87]. It was observed that these mutant bacteria are rapidly destroyed in the presence of light and oxygen with sensitisation by their endogenic chlorophyll, while normal bacteria, containing carotenoids, are completely stable under these conditions. Cells without carotenoids, obtained by being grown in the presence of diphenylamine, behave similarly to the carotenoid-free mutant[88].

Experiments on the protecting action of carotenoids have been performed also on animals. Thus haematoporphyrin was administered to mice with and without the addition of β-carotene before exposure to light. It was found that β-carotene affords a significant protection against the photodynamic effect[89].

+ An erroneous lifetime of $O_2(^1\Delta)$ is quoted in the paper of Merzlyak and Sobolev[80] (p.137) (apparently fortuitously); the lifetime of $O_2(^1\Delta)$ generally accepted at the present time is 2700 s (i.e. 45 min).

Bobrovskii and Kholmogorov[83] investigated sensitised photochemical dehydrogenation reactions in model organic systems with the aim of discovering the mechanism of the photodynamic dehydrogenation of the substrates studied (ionol, phenothiazine, and diphenylamine): the question was whether the dehydrogenation is due to singlet molecular oxygen or takes place as a consequence of the direct interaction of the sensitiser in the excited triplet state with the substrate and subsequent dehydrogenation of the reduced sensitiser by oxygen (Santamaria and Prino[84] rule out the electron transfer mechanism because it presupposes the decomposition of the sensitiser, which has not been confirmed experimentally). Tetrapyrrole pigments (chlorophyll, methyl chlorophyllide, phaeophytin, etc.) were used as sensitisers. The authors established that molecular oxygen in the excited singlet state is in fact the active agent responsible for the dehydrogenation of the substrates employed.

These results led to the conclusion that the destructive agent in the photodynamic effect is singlet oxygen and β-carotene protects living organisms against the photodynamic damage by deactivating ("quenching") singlet oxygen. This deactivation takes place via the transfer of the excess energy of excited singlet oxygen to β-carotene, which results in the formation of oxygen in the ground state and β-carotene in the excited triplet state[22,86,90]:

$$^1O_2 + \beta\text{-carotene} \rightarrow O_2 + {}^3\beta\text{-carotene}.$$

Next it was observed that the quenching takes place effectively when carotenes having nine or more conjugated double bonds are used, while quenching by carotenes having seven or fewer double bonds takes place ineffectively or not at all[91].

Another endogenic quenching agent for singlet oxygen is α-tocopherol. The high efficiency of the quenching of $^1O_2$ by α-tocopherol led many investigators to the conclusion that the biological role of this compound consists in protecting the lipid components of biological membranes against oxidation by a singlet oxygen[92–99] (Section II, 1d and II, 3). It was established that there is a correlation between the activity of α-, β-, γ-, and δ-tocopherols as vitamin E and the efficiency of quenching of singlet oxygen by the latter[92,93].

There exist also exogenic quenching agents for singlet oxygen, for example, certain nickel complexes, which are more stable as a function of time than carotenes, since the latter are gradually consumed in the reaction with $^1O_2$.[100] These complexes can be obtained in forms which are soluble either in water or in lipids. 1,4-Diazabicyclo[2,2,2]octane (DABCO) is a very effective quenching agent. However, it was found that this compound is also an effective trapping agent for hydroxy-radicals ($k \cong 2.5 \times 10^9$ mol^{-1} s^{-1}).[101] Sodium azide is also an effective quenching agent, which is readily soluble in water. (For singlet oxygen quenching agents, see also Section II, 3 below.)

Singlet oxygen can be deactivated not only by a quenching agent but also by a chemical trapping agent ($^1O_2$ acceptor); such deactivation gives rise to the corresponding oxidation products in $^1O_2$ cycloaddition reactions. The trapping agent must then satisfy the following criteria[11,102]: a fairly high rate of reaction with $^1O_2$, specificity in relation to $^1O_2$, the absence of complicating side reactions, and ease of analysis of products. Furthermore, the trapping agent must not participate in the generation of $^1O_2$.

Furans are widely used as trapping agents in biology, but, as has been noted by Foote[100], they are not in practice specific, because many othe oxidants, apart from oxygen, oxidise furans to dicarbonyl compounds. Aromatic hydrocarbons giving rise to *endo*-peroxides are more specific[100]. Dimethylcyclohexane gives rise to a characteristic pair of products of interaction with $^1O_2$; it is not very soluble in water, but may be useful in lipid systems[100]. Limonene, which forms six characteristic products of interaction with $^1O_2$, is analogous to the above compound.

According to Foote[100], the best of the probes for $^1O_2$ known at present is the cholesterol system, proposed by Smith and Stroud[102], who concluded that, among the numerous common cell components which might serve as endogenic trapping agents of $^1O_2$ subject to the criteria mentioned above, only unsaturated aliphatic acids and sterols are promising. They showed that cholesterol (cholest-5-en-3β-ol) as well as two other sterols, namely 5α-cholest-7-en-3β-ol and 5α-lanost-8-en-3β-ol, which are present in mammalian tissues, are effective trapping agents for $^1O_2$, satisfying all the criteria enumerated above. The oxidation of sterols by singlet oxygen give rise to hydroperoxides which are completely different from the hydroperoxides obtained in oxidation by normal oxygen (see also Subsections 1c–1e below).

(b) Photodynamic effect on cells and subcellular components. Many types of photodynamic phenomena have been investigated at the cellular level, for example, the loss by the cell of the ability to form colonies, the arrest and anomalies in cell division, the induction of mutations, a decrease of mobility, a change in the permeability of the membrane and in active transport, the loss of ability to maintain the growth of a bacteriophage, etc.[103] These studies were carried out mainly with unicellular organisms such as bacteria[103–108]. In addition, the photodynamic effect has been investigated on cultured mammalian cells. The cessation of growth and destruction of cell enzymes[109], the cessation of mitosis[110], and destruction of cells[111] were observed. There have been reports of damage to cell organelles, but most of the damage is in the nucleus. Many of the mutagenic and lethal effects investigated, manifested in the presence of photo-sensitisers, are due to conformational changes and the breakdown of strands or the destruction of bases in nucleic acids[112].

Depending on which sensitiser participates in the photodynamic effect, the inactivation of the cell can be of three types[113]: (1) when the cell is attacked from the outside and the sensitiser remains outside the limits of the cell; (2) when the cell is attacked from the inside and the sensitiser is located mainly in the cytoplasm; (3) when the cell is attacked from the inside and the sensitiser is bound preferentially to DNA. In these types of inactivation the sites of damage are respectively the cell surface, the cytoplasm (enzymes, RNA, and other subcellular structures), and DNA. An example of a sensitiser of the first type is provided by the dye Toluidine Blue; many thiazine dyes also belong to this category. The second type consists of xanthenes. Acridine dyes, which can cause genetic changes, belong to the third type.

(c) Genetic effects. The photodynamic effect on biological systems in many instances causes damage to genetic material, resulting in mutation or destruction of the system[112]. Genetic effects (more precisely mutations and chromosomal aberrations) have been observed in many biological systems—from viruses and bacteriophages to higher plants and organisms[112,114–118]. The study of the photodynamic inactivation of bacteriophages has shown that the nucleic acid of the bacteriophage is the target for $^1O_2$:[115,116] it was found that guanine is mainly decomposed[117].

The penetration of the sensitiser (dye) into nucleic acid and its attachment are the necessary stages in this effect[118]. Certain acridine dyes, for example, Acridine Orange, are of special interest in the study of the photodynamic mutagenesis in micro-organisms, viruses, and bacteriophages in view of their high affinity for DNA [119] and the ability of being incorporated between neighbouring bases in the DNA helix.[120]

Before the dye is able to exhibit a sensitising effect, it must initially penetrate through the pellicle of the virus. Incubation with a dye in the dark increases the subsequent effect in the light; consequently the penetration of the dye takes place over an appreciable period[112]. It has been shown[121] that only a dye bound within the virus particle is effective. It has also been demonstrated[118] that the molecules of dyes ineffective in inactivating the bacteriophage can also be bound to the active sites of the nucleic acid. When mixtures of effective and ineffective dyes are used, competition for binding by the ineffective dye lowers the degree of inactivation caused by the effective dye[112].

On photodynamic treatment of DNA, many physiochemical changes in its molecule take place in addition to the selective degradation of guanine. The decomposition of DNA after extensive photodynamic treatment using Acridine Orange has been reported[122]. The authors of this study observed a decrease of the melting point of DNA, which indicates

changes in its conformations. They noted that the decrease of the melting point is proportional to the guanine content in the DNA. A decrease of the thermal stability of the DNA of the T4 phage has been observed[117]; the heat-denatured DNA then underwent photochemical oxidation more readily than the native DNA.

The damage to the skeleton of the DNA molecule on treatment has been investigated[123] by noting the changes in viscosity. The authors[123] suggest that the degradation of neighbouring guanine residues leads to the weakening of the bonds in the DNS skeleton.

(d) Photodynamic damage of membranes. The ability of $^1O_2$ to react with lipids has been confirmed in a number of studies[124–128]. The damage to membranes is believed to be caused by the oxidation of lipids as a result of the photodynamic effect[70,73–80]. It has been shown that aliphatic acid derivatives[129] and cholesterol[130] give rise to photochemical oxidation products in which the double bond has been appreciably displaced towards the allylic position, i.e. the interaction of $^1O_2$ with lipids is a 1,3-cycloaddition with formation of hydroperoxides. The rate of reaction of $^1O_2$ with unsaturated aliphatic acids is approximately 1.5×10^3 times higher than for oxygen in the ground state. The non-conjugated hydroperoxides formed in this reaction indicate the involvement of $^1O_2$ in the peroxide oxidation[102,131,132].

The photochemical oxidation of cholesterol in erythrocytes in the hereditary disease "erythropotic protoporphyria" is a particularly important instance of the peroxide oxidation of lipids by singlet oxygen[94,133]. In this disease 10–100 times more protoporphyrin is observed in the erythrocytes of the patients than under normal conditions. Being an effective sensitiser, protoporphyrin produces singlet oxygen, which damages the erythrocyte membranes. Irradiation of the blood of patients suffering from erythropoetic protoporphyria in the presence of oxygen leads to haemolysis. Singlet oxygen then interacts with the erythrocyte cholesterol, forming cholesteryl hydroperoxide, which induces further haemolysis of the erythrocytes[94,133,134]. It has been observed that internal administration of β-carotene constitutes an extremely effective treatment of this disease[135]. α-Tocopherol also exhibits a protective effect in photohaemolysis[94].

(e) Photodynamic effect on proteins. The first communication about the photodynamic effect on proteins appeared as early as 1903.[136] It was shown that enzymes such as diastase, invertase, and pepsin are inactivated in the light in the presence of eosin. Since then numerous investigations of the photodynamic degradation of proteins, particularly enzymes, have been made[76,77,137]. In all the instances studied the photodynamic damage to the proteins is manifested by a change in the side chains of the aminoacids, but there is no dissociation of peptide or disulphide bonds[76,138].

Of the twenty aminoacids encountered in typical proteins, only five are sensitive to singlet oxygen: histidine, tryptophan, methionine, tyrosine, and cysteine, the first three reacting with $^1O_2$ particularly rapidly[139,140]. The aminoacid residues incorporated in proteins are usually oxidised photochemically more slowly than the same aminoacids in a free state in solution. The incorporation of an aminoacid even in a small peptide can alter profoundly its sensitivity to photochemical oxidation as well as the chemistry of the process[77].

Photodynamic treatment entails a series of physicochemical transformations of proteins as a result of a change in the sensitive aminoacid residues. Changes in solubility[141], surface tension[142], viscosity[143], polarographic behaviour[144], diffusion properties[145], light scattering properties[146], the type of sedimentation[147], optical rotation[147], capacity for assimilation under the influence of proteases[148], binding to metals[149] and coenzymes[150], binding to haem[151], etc. have been noted. The majority of enzymes lose their catalytic activity as a result of photodynamic treatment[76,137], hormones lose their activity[149,152], antigenic properties disappear[137], and the reactivity of antibodies is lost[153].

The aminoacid residues sensitive to photodynamic oxidation change at different rates depending on the reaction conditions and on the protein in which they are incorporated. Thus it is possible to carry out the selective photochemical oxidation of only one type of residue or of a series of residues in proteins using specific sensitisers or reaction conditions. Histidine residues in proteins are known to be sensitive to photochemical oxidation only when the imidazole ring of histidine carries no charge[77]. Thus histidine residues are hardly oxidised photochemically at low pH.[76] Consequently at high pH there is a possibility of the selective oxidation of histidine residues, while at low pH other residues can be oxidised[153–155]. The photochemical oxidation of methionine or histidine forming part of the active centre can be used to investigate the mechanism of enzyme action[156].

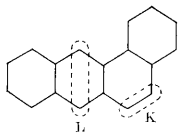
Photochemical oxidation has been used in the conformational analysis of proteins[156–158]. By virtue of the three-dimensional structure of the protein, the residues located on its surface are photo-oxidised at a higher rate than those located at different depths within the molecule. It has been established that the rate constants for the photochemical oxidation of histidine and methionine residues on the surface of the protein are 14 times higher than for those located in the bulk of the molecule[159].

If the sensitiser is associated with the protein, a very high degree of selectivity of the photochemical oxidation of aminoacid residues, located in close proximity to one another as a result of the three-dimensional folding of the protein molecule, is ensured. Chromophores can be bound covalently to specific sites in the protein molecule where they can sensitise the photochemical oxidation of sensitive neighbouring residues. Certain proteins can contain chromophores which are capable of sensitising the photochemical oxidation of aminoacid residues nearby. The required proximity of the sensitiser in the residue depends on the photochemical oxidation mechanism. The photodynamic effect then depends on the distance over which singlet oxygen can diffuse in the course of its lifetime[77]. It has been noted[113] that the possibility of different sites for the generation of $^1O_2$ and for its attack is a unique property of singlet oxygen in the photodynamic effect. Thus the migration of $^1O_2$ through a layer of stearate up to 500 Å thick has been demonstrated[160]. The $^1O_2$ molecule can therefore transport electronic energy over fairly long distances[113].

2. The Involvement of Singlet Oxygen in Carcinogenesis

Numerous attempts have been made to find a correlation between the carcinogenic activity of a compound and molecular electronic factors: charge density[161], electron affinity[162], the ability to form charge-transfer complexes[163], the ability to fluoresce[164], and the energy of the lowest triplet state[165]. The correlation proposed by Schmidt[161] and expressed quantitatively by Pullman and Pullman[166] proved to be best. Pullman and Pullman[166] calculated the charge densities for different carcinogens and observed that carcinogenic activity is

related to the charge density in the K and L regions in polycyclic hydrocarbons:



The K region corresponds to addition to two neighbouring carbon atoms, while the L region corresponds to addition to carbon atoms located in the *para*-positions in the ring. The ease of electrophilic addition in the K and L regions is determined by the charge density at the atoms indicated. The K region can be the site of attachment of the hydrocarbon molecule to the components of the cell[167]. It has been shown experimentally that 1,2 : 5,6-dibenzoanthracene is fixed on the protein of the skin precisely via the K region[168].

It has been demonstrated that the L region of polycyclic hydrocarbons could also be involved in binding to the cell protein[168].

On the basis of the discovery that singlet oxygen can be generated on quenching the excited states of organic molecules (sensitisers) by ordinary molecular oxygen[82,169–171] and bearing in mind the properties of $^1O_2$ as an effective oxidant[12], Khan and Kasha[172] proposed a mechanism of carcinogenesis involving the intermediate participation of singlet oxygen.

According to the model of Pullman and Pullman[166], the two necessary conditions for the carcinogenic properties of polycyclic hydrocarbons are the presence of an active K region and the absence of an active L region. On the basis of their calculations, Pullman and Pullman predicted, for example, the carcinogenic activity of 3,4 : 9,10-dibenzopyrene before this was demonstrated experimentally[173]. Pullman and Pullman were forced to introduce very complex indices into their model in order to account for many experimental observations; nevertheless complete success in predicting carcinogenic activity was not achieved. For example, contrary to the model, 2',3' : 3,4 : naphthopyrene is carcinogenic[172], despite the presence of a very active L region.

According to the theory of Khan and Kasha[172], the initiation of photocarcinogenesis requires that the molecule of the bound polycyclic hydrocarbon should continue to absorb natural sunlight. This type of hydrocarbon molecule, altered as a result of binding to the cell (i.e. the part of the molecule remaining after binding—see Fig. 2), has been called[172] the "optical residue". Thus the bound hydrocarbon molecule plays in this model the role of a bound sensitiser generating singlet oxygen. This mechanism of carcinogenic activity can be represented schematically as follows.

(1) Preliminary binding of the hydrocarbon molecule M to a specific component of the cell Q:

$$^1M + Q \rightarrow {}^1M{-}Q \quad .$$

(2) The excitation by light of the molecule formed:

$$^1M{-}Q \xrightarrow{h\nu} {}^1M^*{-}Q \quad .$$

(3) Intersystem crossing to the triplet state:

$$^1M^*{-}Q \rightarrow {}^3M^*{-}Q \quad .$$

(4) Photosensitised formation of singlet oxygen:

$$^3O_2 + {}^3M^*{-}Q \rightarrow {}^1M{-}Q + {}^1O_2 \quad .$$

(5) The reaction of singlet oxygen with DNA bases initiating the carcinogenic process.

In this model there must be a satisfactory rate of intersystem crossing of the residual molecule from the singlet excited to the triplet state and the energies of the electronic states of the residual molecule must be favourable for the photosensitised formation of singlet oxygen (i.e. the energy of the triplet state of the residual molecule must be $\gg$22.5 kcal mole^{-1}).

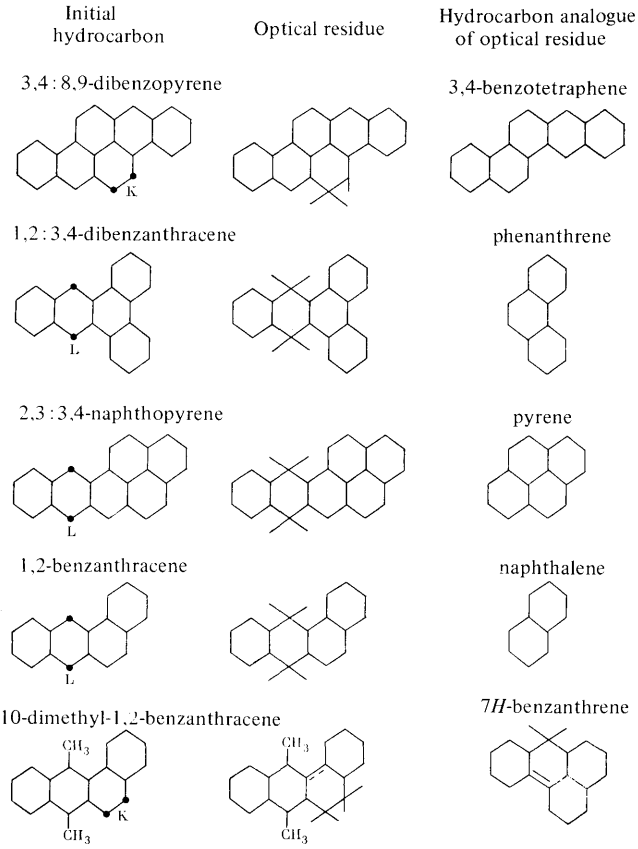


Figure 2. Schematic representation of the optical residues and their hydrocarbon analogues for certain polycyclic hydrocarbons.

Table 1. The absorption of polycyclic hydrocarbons and the analogues of their optical residues after covalent addition to the K or L region[172].

Initial hydrocarbon	λ, nm	Analogue of optical residue	λ, nm
3,4 : 8,9-Dibenzopyrene	480	3,4-Benzotetraphene	410
1,2 : 3,4-Dibenzanthracene	375	Phenanthrene	350
2,3 : 3,4-Naphthopyrene	480	Pyrene	375
1,2-Benzanthracene	390	Naphthalene	317
9,10-Dimethyl-1,2-benzanthracene	390	7*H*-Benzanthrene	350

Khan and Kasha stated that the attachment of the molecule M to the residual cell components via the K or L region shifts two ρ,π orbitals away from the delocalised π-electron system of the polycyclic hydrocarbon molecule as a result of which the optical excitation of the bound molecule requires a higher energy (Table 1), i.e. a blue shift should be observed in the

absorption spectra. The addition via the L region leads as a rule to a more pronounced blue shift of the absorption bands compared with the addition via the K region. This can explain why the presence of an active K region usually favours carcinogenesis, while an active L region normally prevents it. It is noteworthy that the presence of an active L region in the model[172] does not guarantee the absence of carcinogenic activity. Khan and Kasha do not attribute any preferred role to the K or L regions in the induction of cancer; in their model the difference between these regions is determined solely by the optical properties of the residual hydrocarbon from the standpoint of the possibility of the sensitisation of the formation of singlet oxygen.

For example, according to Pullman and Pullman, 3,4 : 8,9-dibenzopyrene (Fig. 2) is a powerful carcinogen, since it has an active K region and has no active L region. Khan and Kasha suggest that the molecule has a carcinogenic activity on the grounds of its optical behaviour, since its addition to a cell component gives rise to an optical residue capable of absorbing sunlight (Table 1). Experimental tests revealed that 3,4 : 8,9-dibenzopyrene is indeed an active carcinogen[172].

The molecule of 1,2 : 3,4-dibenzoanthracene (Fig. 2) has only an active L region and according to the model of Pullman and Pullman should not be carcinogenic. Since addition should displace the absorption spectra to the ultraviolet, Khan and Kasha predicted an insignificant photocarcinogenic activity of this molecule and confirmed this experimentally[172].

The 2′,3′ : 3,4-naphthopyrene molecule (Fig. 2) has no active K region but has a highly active L region and, according to the model for Pullman and Pullman, should not exhibit carcinogenic activity. However, it has been shown experimentally[173] that this compound is a powerful carcinogen. According to the Khan–Kasha model, the residual molecule formed after the addition of the initial molecule via the L region exhibits an optical absorption in the region corresponding to the solar spectrum (Table 1) and for this reason 2′,3′ : 3,4-naphthopyrene should exhibit carcinogenic activity.

Substitution can induce the appearance of carcinogenic activity in molecules which were previously inactive. For example, 1,2-benzoanthracene (Fig. 2) is non-carcinogenic, since the most likely site of the addition of its molecule to the cell component is in the 9,10-positions of the anthracene moiety; such addition shifts the absorption spectrum of the residual molecule to an energically inaccessible region (Table 1). However, the addition of substituents in the 9,10-positions (9,10-dimethyl-1,2-benzoanthracene—Fig. 2) results in the addition of the molecule via the K region rather than the L region and the residual hydrocarbon absorbs sunlight (Table 1). Indeed, 9,10-dimethyl-1,2-benzoanthracene exhibits carcinogenic activity[174].

It is of interest that these planar molecules of polycyclic hydrocarbons—both carcinogenic and non-carcinogenic—are incorporated in DNA molecules[175,176]. Khan and Kasha therefore suggest that the long-lived form of singlet oxygen generated by the residual polycyclic hydrocarbon can diffuse causing oxidation in a very remote section of the DNA chain or can add to certain specific enzyme sites in the cell.

The first experimental test of the theory of photocarcinogenic properties of Khan and Kasha was carried out by its authors[172]. Mice from two colonies (the C_3H breed resistant to cancer of the skin) were smeared with a dilute acetone solution of 3,4-benzopyrene in the standard procedure of the carcinogenic test and were left alone for 5½ months, one colony being placed in a dark room. Twice as many skin tumours appeared in mice living in the normally illuminated room compared with those in the dark room. Control animals living in the dark and normally illuminated rooms did not exhibit this effect.

On the basis of the foregoing, one can conclude that the molecules which are believed to be non-carcinogenic under daylight illumination (the usual wavelength range of solar radiation) can become carcinogenic on irradiation with ultraviolet light.

3. Luminescence in Biological Systems. A New Method of Detecting Singlet Oxygen in a Condensed Phase

Vladimirov[177] began in 1958 studies on ultraviolet luminescence in biological systems; measurements were made with the aid of a photomultiplier cooled with liquid nitrogen and working in the photon counter regime. In 1961 extensive investigations were initiated on the ultraweak glow of tissues and homogenates as well as the glow appearing on oxidation of aliphatic acids (see, for example, Tarusov and Veselovskii[178]).

Among these studies, reactions associated with the action of hydrogen peroxide on biological substrates are especially interesting. An ultraweak glow accompanying the enzymatic decomposition of hydrogen peroxide by catalase, peroxidase, liver homogenates, and yeast and erythrocyte suspensions was observed; the luminescence spectrum was in the range 420–530 nm with maxima at 460 and 510 nm.[177,179] Somewhat later Kachanova and Purmal'[180] confirmed these results, having observed chemiluminescence in the decomposition of H_2O_2 by catalase and metal–amine complexes serving as models of catalase; the emission maximum was observed at approximately 510–520 nm.

In 1966 it was reported[181] that singlet oxygen is formed in the decomposition of hydrogen peroxide by catalase. However, it is claimed[79,182] that singlet oxygen constitutes not more than 0.5% of the entire oxygen formed in this reaction under these conditions. Nevertheless, one can apparently assume that the emission of singlet oxygen is involved in the ultraweak glow occurring in the decomposition of hydrogen peroxide indicated above. The observed region of the $^1O_2$ spectrum includes an emission band at 478 nm, corresponding to the dimolecular transmission[7]:

$$O_2(^1\Delta) + O_2(^1\Sigma) \rightarrow 2O_2(^3\Sigma) + h\nu \quad .$$

Singlet oxygen can then also behave as an intermediate transmitting its excitation energy to other fluorescent molecules[183,184] and can thus participate in luminescence indirectly. There exists yet another mechanism of luminescence involving singlet oxygen. As stated above, $^1O_2$ 1,2-cycloaddition reactions result in the formation of dioxetans, which then decompose into carbonyl-containing fragments, approximately 15% of the fragment molecules being formed in the excited state[185]. This excitation energy can be emitted directly by the fragment or transferred from the latter to a fluorescing molecule[79].

Unfortunately the photomultipliers used to detect the ultraweak glow of plant and animal tissues are insensitive to radiation at wavelengths in excess of 650 nm,[178] while the most characteristic and most intense band in the spectrum of singlet oxygen is at 1270 nm;[11,31,169–171,186] this band corresponds to the $O_2(^1\Delta) \rightarrow O_2(^3\Sigma)$ transition.

The 1270 nm band has been detected by a number of workers in the study of gas-phase systems in the quenching of triplet states of organic compounds by molecular oxygen[11,31,169–171,187,188]. However, in solution it could not be detected for a long time because the lifetime of singlet oxygen in the condensed phase is reduced by eight orders of magnitude compared with the gas phase[7,15,16,184,189,190]. This luminescence band was detected in 1976 by Krasnovskii, Jr.,[191] in air-saturated solutions of various photosensitiser pigments

generating singlet oxygen. The above study[191] was followed by a whole series of investigations by the same author[192–199] who used a sensitive photoelectric technique and solvents (CCl_4, CS_2, and Freon-112) which interact comparatively weakly with $^1O_2$. Aromatic hydrocarbons (anthracene and naphthacene), porphyrins, chlorophylls, bacteriochlorophylls, and phaeophytins were used as photosensitiser pigments. It was found that the spectral region of the flow is independent of the nature of the solvents and pigments.

In all cases a maximum was observed at 1271 ± 1 nm; the emission half-width in spectra recorded using narrow slits (~4 nm) was 18 ± 1 nm, i.e. the glow corresponds to the $^1\Delta$ state of O_2. The luminescence excitation spectra coincided in all cases with the absorption spectra of the photosensitiser pigments. According to Krasnovskii's measurements[192–194], the lifetime of $^1O_2$ in carbon tetrachloride is 28 000 μs, which greatly exceeds the values obtained previously by other workers[202].

Using Krasnovskii's method, it was found that the $^1O_2$ luminescence can be observed in virtually all organic solvents[203] and also in D_2O and H_2O.[195] It is noteworthy that the luminescence quantum yield in D_2O is 400 times smaller than in CCl_4 and amounts to approximately 1.2×10^{-7} and measurement of the $^1O_2$ luminescence in H_2O involves considerable difficulty and a distinct emission maximum could not be observed in H_2O; however, this maximum has been observed largely on diluting D_2O with ordinary water up to the 1 : 1 ratio.

In the study of the above systems Krasnovskii concluded that two processes compete in solutions: photosensitised generation of $^1O_2$ and its quenching in collisions with non-excited sensitiser molecules[192]. He showed that porphyrins, their complexes with zinc, magnesium, and cadmium, chlorophylls, phaeophytins, and retinals effectively generate singlet oxygen[191–198]. Krasnovskii used the quenching of the $^1O_2$ luminescence as a method for investigating the reactivity of $^1O_2$ with respect to various compounds. This method is undoubtedly more accurate than the chemical methods employed hitherto and based on measurements of the rate of consumption of $^1O_2$ trapping agents introduced into the system investigated.

In the course of these experiments it was observed that porphyrins, chlorophylls, and phaeophytins are also effective quenching agents[192–198]. Metal complexes of porphyrins proved to be still more effective quenching agents than metal-free porphyrins; the activity of the quenching agent depends on the nature of the central metal atom and on the structure of the porphyrin ligand. In terms of their activity in the quenching processes, the metals can be arranged in the following sequence:

$$Cu^{2+} < Zn^{2+} < Cd^{2+} \leqslant Mg^{2+} < Fe^{3+} \simeq$$

Porphyrin complexes with Fe^{3+}, Ni^{2+}, and Co^{2+} proved to be the most effective. The quenching rate constant k_q for these compounds are independent of the nature of the ligands and in all cases approach the rate constant for a diffusion-limited process (k_d).

In order to elucidate the quenching mechanism, Krasnovskii measured the rate constants (k_{ox}) for the chemical reaction between $^1O_2$ and quenching agents, which lead to the decomposition of the latter[194,197]. It was found that $k_{ox} \ll k_q$ for porphyrins, chlorophylls, and carotenoids, i.e. the quenching is determined by the physical energy transfer process without the destruction of the quenching agent. For lipids and naphthacene (an aromatic hydrocarbon), $k_{ox} \cong k_q$, i.e. quenching is also determined by the degradative process. On the basis of a comparison of these data, Krasnovskii put forward the hypothesis that two mechanisms of physical quenching are most probable; either direct energy transfer from $^1O_2$ to low-lying levels of the quenching agents or rapid electron exchange in the exciplex between $^1O_2$ and the quenching agents. The first mechanism is possible for quenching agents having energy levels at ≲1 eV (for example, for carotene, bacteriochlorins, and cobalt, nickel, and iron complexes of porphyrins). The second mechanism is more probable for quenching agents without such levels.

The fact that retinals, chlorophylls, and porphyrins are able to generate and quench $^1O_2$ is extremely important for the understanding of photobiological processes such as photosynthesis and vision[197,199]. It is known that the photoreceptors of the retina always contain free retinal which has been shown[197,199] to be an effective photogenerator of single oxygen and can therefore be the cause of the damage of the retina by light. It has been suggested that in the system protecting photoreceptors from singlet oxygen the leading role is assumed by α-tocopherol,[204] whose content in the outer segments of the rods in the retina is 5–10 times higher than in the other biomembranes.

Somewhat later than Krasnovskii's investigations, Khan and Kasha also detected the $^1O_2$ luminescence at 1270 and 1580 nm in liquid solutions[200,201]. They used an extremely sensitive infrared spectrometer with a thermal electrically cooled PbS detector. A much weaker band at 1580 nm corresponds to the $^1\Delta(v' = 0) \rightarrow {}^3\Sigma(v'' = 1)$ transition. $^1O_2$ was generated via photosensitisation of the solution and also chemically in the reaction of hydrogen peroxide with sodium hypochlorite. Benzophenone, haematoporphyrin, etc. were used as the sensitisers, while the solvents were perfluorohexane, Freon, CCl_4, and water.

The $^1O_2$ photoluminescence can be used to detect the ability of various substances to generate $^1O_2$ photochemically and also to estimate the efficiency of this generation process. Thus this new method of detecting singlet oxygen can be very important not only for the study of natural biological processes but also for the investigation of the effect on natural systems of exogenic components, which is associated particularly with problems involving the care of public health.

III. SINGLET OXYGEN IN POLLUTED CITY AIR

The possibility that singlet oxygen plays a decisive role in the formation of photochemical smog in the lower atmosphere was first examined in the 1960's.[205,206] Later this problem was studied in greater detail[207–209]. As a result of prolonged studies of the mechanisms of the formation and decomposition of photochemical smog, Pitts and coworkers[210,211] concluded that singlet oxygen $O_2(^1\Delta)$ and (or) $O_2(^1\Sigma)$ in polluted city air participates in chemical processes as an oxidant. These authors suggested that certain hitherto inexplicable atmospheric chemical and possibly biological effects could be attributed to singlet oxygen.

The ways in which singlet oxygen is formed in polluted city air are examined below. Five mechanisms, explaining the formation of singlet molecular oxygen in polluted city air have been proposed[207]. The first four mechanisms as well as the contribution of each to the production of singlet oxygen are illustrated in Table 2.

1. Direct Absorption of Sunlight

After a series of experiments on a mixture of air and tetramethylethylene in an apparatus with a long optical path, Pitts and Coomber[207,208] concluded that the direct absorption

of visible radiation in the range 550–770 nm by normal oxygen leads to the formation of singlet oxygen $O_2(^1\Sigma)$ (the $^3\Sigma + {}^1\Sigma$ transition at 762 nm), which is rapidly deactivated to the $^1\Delta$ state. $O_2(^1\Delta)$ then reacts with tetramethylethylene and the corresponding hydroperoxide is formed. Furthermore, the absorption at 1270 nm leads to the formation of $O_2(^1\Delta)$. However, since these transitions are formally forbidden, the contribution of this mechanism to the overall production of singlet oxygen in the atmosphere is very small.

Table 2. The formation of $^1O_2$ in polluted city air[220].

Source of $^1O_2$	w^*, 10^{-8} h^{-1}
Direct optical absorption	$<9.2\cdot10^{-3}$
Photosensitisation	80
$O_3+h\nu\rightarrow{}^1O_2+O^*$	0.1
$NO+O_3\rightarrow NO_2^*+{}^1O_2$	1140

*Here w is the rate of production of $^1O_2$.

2. Energy Transfer from Organic Molecules in the Triplet State to Oxygen in the Normal State (Photosensitisation)

A considerable amount of singlet oxygen is obtained by the direct transfer of electronic energy to the oxygen molecule from the molecules of an organic pollutant in the triplet state[207,210,212]. This mechanism is identical with Kautsky's mechanism of laboratory photosensitised oxidation reactions[82]. Spectroscopic studies by a number of workers showed that $O_2(^1\Delta)$ is produced by the gas-phase energy transfer to oxygen from irradiated gaseous aromatic hydrocarbons with (π,π^*)-triplet states (for example, benzene and naphthalene)[169,171,213] and carbonyl compounds with (n,π^*)-triplet states, for example benzaldehyde[208,214]. Energy transfer has also been observed for heterogeneous gas–solid and gas–liquid systems[215].

3. The Photolysis of Ozone

When ozone is irradiated with ultraviolet light, it undergoes photodissociation to an electronically excited oxygen atom $O(^1D)$ and an oxygen molecule in the singlet state[216,217]:

$$O_3 + h\nu_{260\ nm} \rightarrow O(^1D) + O_2(^1\Delta \text{ or } {}^1\Sigma) \quad .$$

This mechanism is particularly important in the upper layers of the atmosphere[218]. In the lower layers it is less important than the energy transfer mechanism[219,220].

A significant consequence of the formation of $O(^1D)$ atoms in this reaction is its involvement in the subsequent energy transfer process:

$$O(^1D) + O_2 \rightarrow O_2(^1\Sigma) + O(^3P) \quad .$$

This results in the formation of two oxidising species; singlet molecular oxygen and atomic oxygen in the ground state[207].

4. Exothermic Chemical Reactions

An important exothermic reaction in a polluted atmosphere giving rise to $^1O_2$ is that between nitric oxide and ozone[207], the rate constant for which is $k = 1.2 \times 10^7$ litre mol^{-1} s^{-1}:[221]

$$NO + O_3 \rightarrow NO_2^* + {}^1O_2 \quad .$$

Furthermore, it has been suggested[222,223] that ozone might react similarly with a series of other substances polluting the atmosphere, such as sulphides, with formation of singlet oxygen. It is known that similar processes in the condensed phase do lead to the formation of singlet oxygen[207].

The high reactivity of peroxyacetyl nitrate (PAN) in chemical and biological systems *in vitro* and *in vivo* is known. It has been shown that PAN is one of the main causes of the irritation of the eyes and the damage to plants by polluted city air[224–226]. Studies on simpler systems have shown that PAN can act both as an oxidising and an acetylating agent[227,228]. Alkaline hydrolysis of this compound leads to the formation of singlet molecular oxygen[207,229,230]:

$$CH_3C(=O)OONO_2 + 2OH^- \longrightarrow CH_3C(=O)O^- + H_2O + NO_2^- + {}^1O_2 \quad .$$

Thus singlet oxygen must be regarded not only as a possible oxidant acting on the environment but also as an intermediate in the oxidation process.

5. Charge-Transfer Complexes between a Hydrocarbon and Oxygen

Coomber and coworkers[208,209] proposed a fifth way leading to the formation of singlet molecular oxygen, which is as follows. When ultraviolet light is absorbed by short-lived olefin–oxygen or aromatic hydrocarbon–oxygen collision complexes, charge-transfer complexes are formed between the electronically excited hydrocarbon (electron donor) and oxygen (electron acceptor) in the ground state. These complexes can then decompose, giving rise to singlet oxygen and the triplet hydrocarbon[207–209].

When a charge-transfer complex is formed between a hydrocarbon and oxygen, an additional intense absorption band, corresponding to charge transfer, appears in the absorption spectrum of the hydrocarbon, which results in an increase of the intensity of the long-wavelength edge of the hydrocarbon absorption band and in addition the band undergoes a shift towards longer wavelengths ("red shift") (see, for example, Tsubomura and Mulliken[231] and Shinkarenko[232]). The fact that the long-wavelength edge of the absorption band of hydrocarbons becomes more intense and undergoes a bathochromic shift in the presence of oxygen under illumination is extremely important for atmospheric photochemistry. It was believed previously that the presence of many of the polluting compounds in the environment is unimportant because they do not absorb light in the region corresponding to the solar spectrum. However, the red shift of the absorption band suggests that some of these compounds do indeed absorb in the visible region and therefore participate in the photochemistry of polluted city air.

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