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Invited Review

Photosensitization Reactions of Biomolecules: Definition, Targets and Mechanisms

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ABSTRACT

Photosensitization reactions have been demonstrated to be largely responsible for the deleterious biological effects of UV and visible radiation, as well as for the curative actions of photomedicine. A large number of endogenous and exogenous photosensitizers, biological targets and mechanisms have been reported in the past few decades. Evolving from the original definitions of the type I and type II photosensitized oxidations, we now provide physicochemical frameworks, classifications and key examples of these mechanisms in order to organize, interpret and understand the vast information available in the literature and the new reports, which are in vigorous growth. This review surveys in an extended manner all identified photosensitization mechanisms of the major biomolecule groups such as nucleic acids, proteins, lipids bridging the gap with the subsequent biological processes. Also described are the effects of photosensitization in cells in which UVA and UVB irradiation triggers enzyme activation with the subsequent delayed generation of superoxide anion radical and nitric oxide. Definitions of photosensitized reactions are identified in biomolecules with key insights into cells and tissues.

INTRODUCTION

During the past few decades, reports of photosensitization reactions of biomolecules, including proteins, lipids and nucleic acids, became available together with their implication in various biological effects such as cell lethality, carcinogenesis, aging, as well as in light-based medical treatments. Details of the photosensitization mechanisms have increased, but key steps in these processes are only found scattered in the scientific literature and are usually disregarded in several proposed mechanistic explanations. Indeed, the effects of UVA/visible light radiation in human skin can only be understood by considering both oxygen-dependent and oxygen-independent sensitized reactions with target molecules. This concerns, in particular, type I and type II photosensitization oxidation mechanisms that were initially proposed by Foote (1) and recently partly revisited (2). We avoid the increase in the numerical types of mechanisms, for example, type III or IV mechanisms, which have recently been proposed in the literature to classify O_2-independent photosensitized reactions. The main aim of the present survey was to critically review, in an extended manner, all identified photosensitization mechanisms of biochemical molecules, by providing a few relevant examples. We also cope with the increasing need to clarify the relevant mechanisms of photosensitization of nucleic acids, proteins and unsaturated lipids, inferred from model studies and to also evaluate the subsequence cellular responses. Direct and indirect evidence provides clues to the roles of intermediates. Often reliant on model reactions, reaction patterns and definitions are needed. Better articulated definitions of biomolecules would be of benefit to the field. The review article is completed by the presentation of photosensitized reactions that were identified in cells/animal tissues.

Unifying definitions of biological photosensitization reactions

Definitions supplied by studies of simple organic reactions are a starting point that needs a more sophisticated approach to be expanded to biological systems, which have their specific boundary conditions. We propose that in biological systems, the terms photosensitization reaction (or process), photosensitized reaction (or process) and, simply, photosensitization should be considered synonymous and can be defined as a process by which a chemical change occurs in one compound, the substrate or target, as a result of initial electronic absorption of UV/visible radiation by the photosensitizer or just the sensitizer. While the substrate is always consumed in the process, the photosensitizer may or may not be consumed, depending on the mechanism.

Photosensitization has different meaning from photocatalysis, and the words should not be used as synonyms. According to the definition of photocatalysis and photocatalyst given in the IUPAC "Glossary of terms used in photochemistry" (3), a

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photocatalyst absorbs radiation in the process and always regenerates itself after each cycle of interactions with the reaction partners. The photosensitizer always fulfills the first condition, while the second does not. This differentiation between both terms can be controversial, and there are authors that considerer that the photosensitizer must be recovered in the process, that is, each molecule of photosensitizer must convert many substrate molecules into photoproducts. However, we are inclined to accept a definition more extensive and pragmatic. In fact, many compounds widely accepted as photosensitizer are consumed in the photochemical process. Although the word photosensitizer is not synonymous with heterogeneous photocatalysis, nanoparticles can function as photosensitizers, for example, in dye-coated particles in the photoinactivation of microorganisms.

Photosensitizers can be endogenous or exogenous compounds. In the former group, many natural heterocyclic compounds can act as photosensitizers, such as porphyrins, flavins, pterins and lumazines (4). Some products of oxidation of normal components of the cells can be added to this group; for instance, some products of oxidation of Trp (5–7). Although the endogenous photosensitizers are usually present at very low concentrations and their photoactivity is limited, they explain part of the deleterious effects of UVA and visible solar radiation. In addition, they can accumulate under certain pathological situations, increasing the photodamage. Among the latter group, a large number of xenobiotics can be found, mainly pharmaceutical drugs and pollutants. Apart from the harmful effects induced in biological systems, photosensitization reactions can be beneficial for medical and environmental applications, such as photodynamic therapy to destroy tumors (PDT) (8–10), photodynamic inactivation of microorganisms (PDI) (11–13) and contaminant photodegradation (14–17). A large number of compounds have been designed to act as photosensitizers for such applications. It is worth commenting on the term "photodynamic." This word involves the participation of O_2, and therefore, PDT and PDI expressions, which are widely used in medicine, pharmaceutical sciences and microbiology, refer to processes in which the appropriate combination of electromagnetic radiation, a photosensitizer and O_2 are used to destroy a cell target (cancer cell or pathogenic microorganism).

The group of biological molecules that may be the targets of photosensitization reactions is large and diverse. However, considering their susceptibility, concentration in living organisms and the biological consequences induced by the photosensitized modifications, it is worth mentioning among the main targets a few amino acids [tryptophan (Trp), tyrosine (Tyr), histidine (His), methionine (Met), cysteine (Cys), etc.], nucleobases [guanine (G), adenine (A), thymine (T), cytosine (C)] and unsaturated fatty acids. These biomolecules can be damaged by photosensitization reactions when they are free, part of macromolecules including proteins or nucleic acids, or a supramolecular structure, such as a biomembrane.

GENERAL CLASSIFICATION OF THE MECHANISM OF PHOTOSENSITIZATION REACTIONS

The initial physical event in a photosensitization reaction is the absorption of a UV/visible photon by the photosensitizer. For most organic photosensitizers, the resulting singlet excited state undergoes intersystem crossing to yield a longer lived triplet excited state. The first bimolecular step of the process is the reaction of the singlet or triplet excited state of the sensitizer with the substrate or with dissolved molecular oxygen (O_2) (Scheme 1). Photosensitized reactions that apply to biomolecules may be generally classified as oxygen dependent and independent. It is important to emphasize that this classification is not based on whether the excited sensitizer reacts with O_2, but whether O_2 is needed in the overall process. That means that in photosensitized oxidations, O_2 may react with the electronically excited photosensitizer or participate in a secondary step, such as the reaction with radicals issued from the photosensitizer or substrate. The well-documented type I and type II mechanisms, originally defined by Foote (1) and recently revisited (2), are mainly restricted to photosensitized oxidations (oxygen-dependent processes), with exceptions that will be discussed later.

Type I photosensitized reactions involve electron transfer and lead to the initial formation of radicals and the participation of O_2 in subsequent steps involved in the oxidation process. A type I mechanism is initiated by an electron transfer reaction between the excited sensitizer ($Sens^*$) and the substrate. This redox reaction can take place in both directions, that is, the substrate can be reduced or oxidized; however, oxidation is almost always observed for biomolecules (reaction 1). The initial process is an electron transfer from the biological target to $Sens^*$ giving rise to the corresponding pair of radical ions ($Sens^{\bullet -}$ and $S^{\bullet +}$), which in turn, can be in equilibrium with their corresponding neutral radicals [$SensH^{\bullet}$ and $S(\text{-H})^{\bullet}$]. However, it is worth mentioning that several electron-transfer processes in biological systems occur coupled to a proton transfer. Therefore, formally, one should also consider proton-coupled electron transfer (PCET), in which the electron transfer reaction is affected by the concomitant transfer of one or more protons. In effect, PCET can be a simple hydrogen atom transfer (HAT), when both the electron and the proton are transferred from the same bond.

Alternatively, the other first bimolecular reaction that can initiate a type I mechanism is reaction 2, in which excited sensitizer reduces O_2 leading to the formation of superoxide anion radical ($O_2^{\bullet -}$). In the initial classification proposed by C. S. Foote (1), reaction 2 was considered as a type II mechanism because the excited sensitizer reacts with O_2, as in the case of $^1O_2$ formation. However, we classify this reaction as type I because we define type I on the basis of the formation of radicals.

On the other hand, type II mechanism involves an initial energy transfer from the triplet excited state of the sensitizer to dissolved O_2, which is in its ground triplet state [$O_2(^3\Sigma_g)$ (denoted as O_2)], yielding singlet molecular oxygen [$O_2(^1\Delta_g)$, denoted throughout as $^1O_2$], the lowest excited state of molecular oxygen (reaction 3) (18–23). Molecular oxygen in this activated (metastable) state is far more reactive than in the ground state.

Several mechanisms can be involved in the oxygen-independent photosensitization. One of the most relevant photosensitized reactions involves energy transfer from the excited sensitizer to the substrate (triplet–triplet energy transfer, TTET) (reaction 4). Once in the excited state, the substrate may react with a vicinal molecule to form a dimeric photoproduct according to a [2 + 2] photocycloaddition reaction. A second group of oxygen-independent reactions give rise to the formation of photoadducts in which the sensitizer and the substrate are covalently bound (reaction 5). Although different mechanisms can be involved in the formation of photoadducts, the [2 + 2] cycloaddition (photocycloaddition) is perhaps the most important.

In reactions 1, 4 and 5, the excited sensitizer directly reacts with the substrate and therefore can be assumed as contact-dependent processes, that is, an encounter between the two molecules occurs. In fact, these reactions are in cells only efficient when the photosensitizer and the target are in close vicinity. If the reaction is a dynamic process, the rate is controlled by diffusion. In contrast, if there is a previous association between the two molecules, the process is not limited by diffusion and can be much faster. That is why, in some cases, the association of the sensitizer in its ground state with the substrate may make much more efficient a contact-dependent photosensitized process. On the other hand, reactions 2 and 3 are contact-independent processes, that is, the photosensitization does not require an encounter between the excited sensitizer and the substrate and the reaction can occur even when both species are physically separated if the reactive intermediate is able to reach with the target molecule. In particular, $^1O_2$ that only reacts significantly with dedicated biomolecules (*vide infra*) may diffuse to a certain extent in cells before reaching its targets.

Despite much progress, details underlying the definitions are difficult to dissect. There is some ambiguity and confusion in the definitions and classification of the mechanisms that we would like to clarify. Sometimes, it is accepted that all possible mechanisms of photosensitization are divided into type I and type II, but it is important to emphasize that this is just the classification of the processes involving O_2. The processes initiated by reactions 4 and 5 are also photosensitization mechanisms that however do not fall within the definition of type I and type II mechanisms. Other mistaken idea is that photosensitization always takes place involving a reactive oxygen species (ROS) and that $O_2^{\bullet-}$ and $^1O_2$ are the intermediates responsible for the photodamage caused by type I and type II photooxidations, respectively. Although $^1O_2$ is in fact the oxidizing species involved in type II mechanism, $O_2^{\bullet-}$ plays a minor role if any since it does not exhibit significant reactivity toward most biomolecules (*vide infra*). The chemical changes in type I photooxidations are mainly due to the reactions undergone by organic radicals [$S^{\bullet+}$/S(-H)$^\bullet$ in reaction 1] that further react, almost always, with the participation of O_2.

The mechanisms operating in a given photosensitized process and their rates depend on many factors, and it is usual that several competitive pathways involving different mechanisms participate. After the initial bimolecular reactions listed in Scheme 1, many different subsequent reactions can take place, which depends on the experimental conditions and the nature of the reactive species generated, in the contact-dependent processes [$S^{\bullet+}$/S(-H)$^\bullet$, S^*], and the reactivity of the substrate toward the reactive intermediate ($O_2^{\bullet-}$, $^1O_2$), in the case of contact-independent processes.

In the next sections, some typical subsequent reactions that take place after the initial bimolecular reactions (Scheme 1) are given for each type of mechanism. Additionally, a few selected examples of photosensitized reactions for which relevant mechanistic insights were gained from experimental and/or theoretical studies on either model compounds or preferentially the entire biomolecules are provided. In no way, the next sections will provide a complete and exhaustive review of the countless photosensitized reactions reported in the literature, but they will shed light on the diversity and complexity of photosensitization-mediated degradation pathways of biomolecules (nucleic acids, proteins and unsaturated lipids) that may induce adverse biological effects or are the bases of beneficial medical and environmental applications.

TYPE I PHOTOSENSITIZED OXIDATIONS

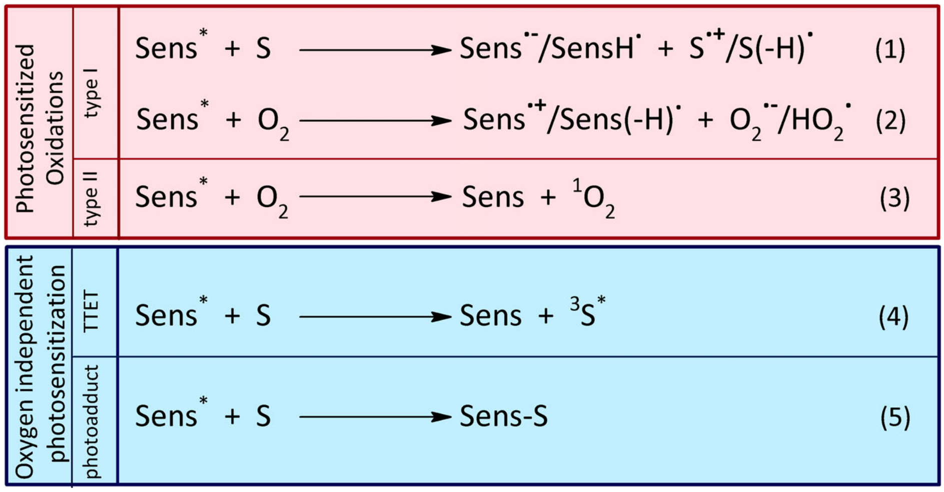
General features

A considerable portion of the transformations induced by excited states occurs by type I photosensitized oxidations. Generally speaking, upon light absorption with the transient formation of excited state species, stronger oxidizing and stronger reducing agents are formed. Whether or not the excited state will engage in a redox process (reaction 1) depends on many factors, including the molecular contact of the excited state with biological targets and the energetics and the relative rate of the electron transfer reaction in comparison with other photophysical processes, in particular deactivation of the triplet excited state by energy transfer to O_2 to form $^1O_2$ (reaction 3). The possibility of an electron-transfer reaction can be estimated by considering the thermodynamic tendency of the molecules to receive or donate electrons (redox equilibria). Consequently, excited-state redox potentials have considerable utility in predicting type I redox reactivity (*vide infra*) (24–26).

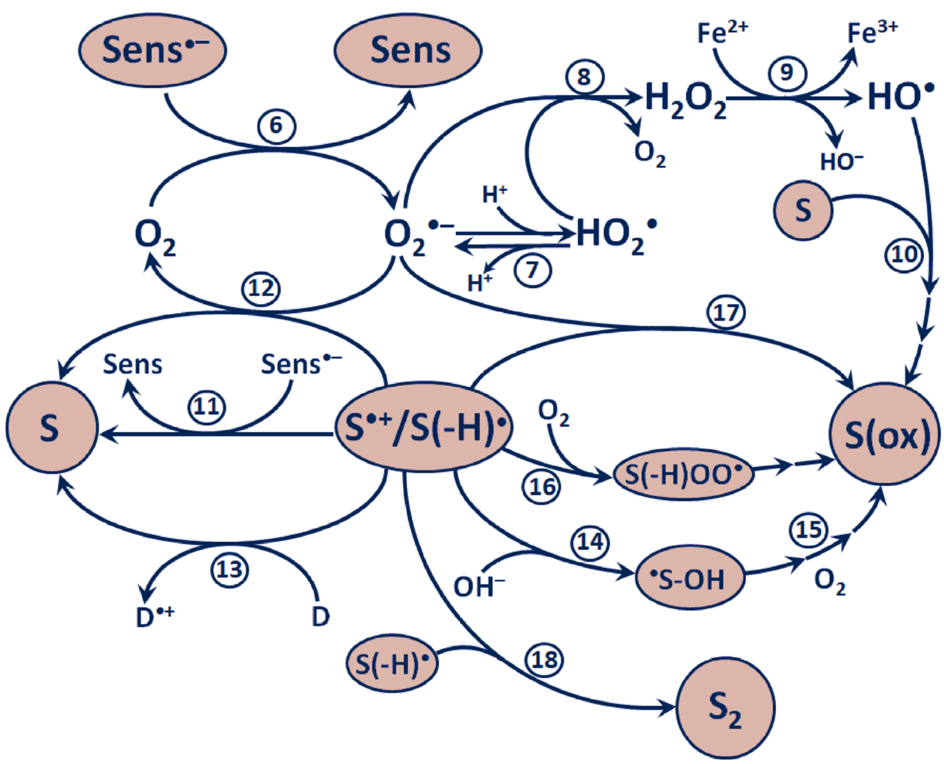
After the initial one-electron oxidation (reaction 1), both radicals formed participate in a complex set of competitive pathways, which are summarized in Scheme 2. In general, the photosensitizer radical anion reacts with O_2 to regenerate the sensitizer and to form $O_2^{\bullet-}$ (reaction 6) (27). This represents the main source of $O_2^{\bullet-}$ in photosensitized reactions, being much more relevant than the direct reduction of O_2 by excited sensitizer (reaction 2). $O_2^{\bullet-}$ that is predominant at physiological pH is in equilibrium ($pK_a = 3.6$) with its protonated form $HO_2^\bullet$ (reaction 7) and disproportionate to hydrogen peroxide (H_2O_2) (reaction 8), another low reactive ROS. This compound, as well as $O_2^{\bullet-}$ and $HO_2^\bullet$, does not exhibit significant reactivity toward most biomolecules (28). However, H_2O_2 upon reduction triggered by transition metals (Fe^{2+}, Cu^+) or ascorbate is able to generate highly reactive hydroxyl radical ($HO^\bullet$) (reaction 9) that reacts with biological substrates at the site where it is produced (reaction 10).

The radical formed from the one-electron oxidation of the target molecule [$S^{\bullet+}$/S(-H)$^\bullet$] may undergo a large number of reactions (Scheme 2). The predominant pathway depends on the experimental conditions (concentrations, pH, etc) and on the chemical nature of the radical. It is worth mentioning that reaction 1, that initiates most type I processes, does not involve the participation of O_2. Almost always, as discussed later, in the series of subsequent chemical reactions occurring from the initial radical to the final product, O_2 is involved in at least one step.

The recovery pathways leading back to the original substrate S compete with reactions that lead to the formation of oxidation products. Among the former group, the recombination of the radicals formed in the first step may recover both the substrate and the sensitizer (reaction 11) (29). This pathway is frequently the predominant one in the absence of O_2, and consequently, no net substrate consumption is observed under anaerobic conditions, even when radicals are formed, that is, reaction 11 counteracts the initial one-electron oxidation (reaction 1). The substrate can



Scheme 1. First bimolecular events for each type of mechanism. Sens*: sensitizer excited state; S: substrate.



Scheme 2. Type I reactions. Subsequent reactions underwent by the initial radicals formed in reaction 1.

also be regenerated by reduction by $O_2^{\bullet -}$ (reaction 12) or by an electron donor present in the medium, such as a thiol (reaction 13).

Many reactions of the substrate radical can initiate pathways that eventually give rise to oxygenated products. Besides the deprotonation, a common reaction of the radical cation ($S^{\bullet +}$) is hydration that often yields C-carbon centered radicals (reaction 14). Both neutral radicals (S(-H)$^\bullet$ and $^\bullet$S-OH) may be ranged into several distinct reactive intermediates with different chemical reactivity according to the target molecule. In particular, $^\bullet$S-OH may further react with O_2 by either addition or by one-electron oxidation (reaction 15), whereas S(-H)$^\bullet$ may also further react with O_2 (reaction 16) or with $O_2^{\bullet -}$ (reaction 17). In Scheme 2, S (ox) represents a vast and heterogeneous group of oxidized substrates, most of which are oxygenated, such as those arising from the reactions of O_2 or $O_2^{\bullet -}$ with the radicals formed in the initial step (reaction 1). It is worth mentioning that S(ox), by no means, represents final and stable products. In contrast, S(ox) can be thermally or photochemically unstable or can undergo further photosensitization at least in model systems giving rise to countless pathways with rates depending on the environmental conditions.

Finally, two S(-H)$^\bullet$ can react to give rise to a dimer S_2 (reaction 18). In the sequence of reactions, O_2 does not participate.

In some cases, O_2 is needed in the overall process to avoid the recovery of the substrate via reaction 11, that is, O_2, by quenching $Sens^{\bullet-}$ (reaction 6), prevents the recombination of radicals and favors the reaction between two $S(-H)^{\bullet}$ (30). In other cases, O_2 is not needed at all and S_2 can be formed under anaerobic conditions, even with higher efficiencies than in the presence of O_2 (31). Whether O_2 favors or hinders the photosensitized formation of S_2 through these mechanisms depend on many factors. In particular, it depends on the result of the rate of the competitive pathways for a given system. To give just some simple examples, if the recombination reaction 10 is fast and the reactions 13-16 are slow, the formation of S_2 will be favored in the presence of O_2; in contrast, if reaction 10 is slow and reactions 13-16 are fast S_2 will be favored in the absence of O_2. In addition, $S^{\bullet+}/S(-H)^{\bullet}$ can react with radicals coming from a different substrate giving rise to the formation of adducts S1-S2. Apart from O_2 concentration, sensitizer properties and other conditions affect the efficiency of the dimerization of the substrate. Indeed, high sensitizer and substrate concentrations and high radiation intensity will increase the concentration of radicals and favor the dimerization.

The case that we have just considered, radical-mediated dimerization of the substrate (reaction 18), can be classified as type I photooxidation or not. Strictly, the dimerization is an oxidation, but without the incorporation of oxygen atoms to the products. Therefore, if a type I reaction needs O_2, this processes should be excluded from this category. If type I means oxidation initiated by generation of radicals, dimerization can be accepted within this group. This issue is controversial and there is no consensus in the literature. The discussion remains open on this point with the hope of coming to an agreement in the near future.

At this point, it is worth analyzing the thermodynamics of type I mechanism. Considering direct oxidation by photosensitization, the tendency of a photosensitizer to act as a photochemical oxidant can be quantified in terms of its one-electron pseudoreduction potential ($E'_{ox}(Sens^{*}/Sens^{\bullet-})$) (24) (Equation 19). The feasibility of the electron-transfer process will also depend on the one-electron reduction potential of the substrate (S) and on the network required to bring products and reactants close together (Δw) (Equation 20) (25, 32).

$$E'_{ox}(Sens^{*}/Sens^{\bullet-}) = E'_{1/2}(Sens/Sens^{\bullet-}) + \Delta E(eV) \quad (19)$$

$$\Delta G(eV) = E_{ox}(Sens{*}/Sens^{\bullet-}) - E'_{1/2}(S/S^{-}) + \Delta w(eV) \quad (20)$$

Values of Δw are < 0.1 eV in water or one or two orders of magnitude smaller than the pseudoreduction potentials of the photochemical oxidants. Consequently, Δw is negligible (24). Therefore, the feasibility of a type I photooxidation process can be estimated by comparing the pseudoreduction potentials of the photochemical oxidants with the formal reduction potential of the S. Table 1 lists E'_{ox} ($Sens^{*}/Sens^{\bullet-}$) values for a series of important photochemical oxidants, organized into two general categories: endogenous, *that is*, those photosensitizers that are naturally present in cells and are responsible for photosensitization phenomena induced by the direct light exposure in living organisms and exogenous, *that is*, natural or synthetic molecules that are not found in human skin and are typically used as photosensitizers in medical treatments.

References Table 1 (33–76).

In order to facilitate the discussion and, in many cases to compensate for the lack of data on triplet state energies, we only show the estimated (0,0) energy levels for the singlet excited states (Table 1). Although both singlet and triplet excited states can engage in type I reactions, singlet excited states have a higher excitation energy (by 0.2-0.6 eV) compared with the lowest triplet excited state (77). In pterins, for example, the lowest singlet and triplet excited states are respectively 3.1 and 2.5 eV above the ground state (78). However, the triplet state has a much longer lifetime and in practice the triplet excited pterin can diffuse much further to encounter the electron acceptor, while the singlet excited state will react only if it is already in close proximity to the substrate.

Any molecule that can accept an electron is a potential photochemical oxidant. Excitation of a molecule in the UV or visible increases the photosensitizer oxidizing tendency by 4 to 1.5 eV, transforming poor ground state oxidants, such as the purine and pyrimidine bases, into strong excited state oxidants. Photoactive amino acids (Phe, Tyr, Trp) do not have stable one-electron reduction curves and, consequently, cannot work as photochemical electron acceptors. On the other hand, these amino acids can be oxidized at relatively small potentials and consequently, work as strong photochemical electron donors (Table 1). Several enzymatic cofactors, for example, flavins and pterins are known to behave as endogenous photosensitizers (79–81). Their excited states become strong oxidant agents with pseudo reduction potentials in the order of 2–3 V. The same range of oxidizing power is found for endogenous pigments like lipofuscin and melanin. Synthetic photosensitizers employed in PDT usually absorb in the visible range and have pseudoreduction potentials smaller than 2 V. Nevertheless, for molecules that have formal reduction potentials between −0.5 and +0.5, which includes different types of photosensitizers like phenothiazinium ions, chlorins, bacteriochlorins, porphyrazins, their excited states will still have pseudoreduction potentials above 1 V.

The other important variable in the Equation 20 is the reduction potential of the substrate (Table 2). The tendency of a biomolecule to donate an electron to a photochemical oxidant will increase with the decrease in the reduction potential of their respective one-electron oxidation product. According to this, in the case of nucleobases and amino acids, the tendency to undergo one-electron oxidation is G > A > T, C (82–85) and Tyr > Trp > His (86, respectively. Note that lipids, especially poly-unsaturated lipids, are the easiest to oxidize, with $E^{\circ\prime}$ close to those of anti-oxidants such as tocopherol and ascorbic acid (Table 2). Even saturated lipids or any other molecule with allylic or *bis*-allylic hydrogens (such as carotenoids, for example), will have $E^{\circ\prime}$ below 1 V, making them possible targets for most of the sensitizers mentioned in Table 1. Several amino acids (cysteine, tyrosine, tryptophan), lipid hydroperoxides (Table 2) and small redox-active molecules (Table 2), hydrogen peroxide and nitrite (Table 3) are prone to be oxidized by the majority of the endogenous and exogenous photosensitizers. E'_{ox} ($Sens^{*}/Sens^{\bullet-}$) values for porphyrins are well below 1V, meaning that they will not have enough driving force to abstract electron from most of the biological targets (Table 2). No wonder that studies performed in membrane mimetic systems seem to indicate that porphyrin sensitizers only engage in type II photosensitized oxidation reactions (86).

References Table 2: (87–92)

References Table 3: (93–98)

A second type I process, not included in Scheme 1 because it is less common, is the direct reduction of the substrate by the

Table 1. Relevant photophysical properties of endogenous and exogenous photosensitizers and their respective pseudoreduction potentials when acting as electron acceptors or electron donors.

			Photochemical electron acceptor		Photochemical electron donor	
Photosensitizer	λ (nm) [eV]*	S_Δ[†]	$E_{1/2}$ (V), SHE	E'_{ox} ($PS^*/PS^{\bullet-}$)[‡]	$E_{1/2}$ (V), SHE	$-E'_{red}$ ($PS^{\bullet+}/PS^*$)[§]
Endogenous						
Thymine	300 [4.1]	0.07 (33)	−1.1 (34)	3.0	2.1	2.0
Adenine	289 [4.3] (35)	0.1 (33)	−1.2 (36)	3.1	1.9	2.4
Cytosine	300 [4.1]	0.03 (33)	−1.1 (34)	3.0	2.1	2.0
Guanine	336 [3.7] (35)	<0.005 (33)	−1.2 (36)	2.5	1.5	2.2
Phe	267 [4.6]	0.065 (37)			0.3 (38)	4.3
Tyr	288 [4.3]	0.138 (37)			0.9 (39)	3.6
Trp	307 [4.0]	0.062 (37)			1.0 (39)	3.0
Lipofuscin	425 [2.9]	0.1 (40)	0 (41)	2.9	0	2.9
Melanin	425 [2.9]	0.02 (42)	±0.02 (43)	2.9	0.2	2.7
Pterin	400 [3.1]	0.2 (44)	−0.5 (45)	2.6	0.3	2.8
Riboflavin	490 [2.5]	0.5 (46)	−0.25 (47)	2.3	−0.2	2.7
Chlorophyll[‖]	650 [1.9]	0.6 (48)	−0.7 (49)	1.2	0.7	1.2
Bac-chlorophyll[¶]	665 [1.9]	0.5 (50)	−0.7 (51)	1.3	0.7	1.2
Porphyrins[#,**]	~610 [2.0]	0.7 (52)	−1.5 (53)	0.5	1.1 (54)[††]	0.9
Exogenous						
Coumarin	365 [3.4] (55)	0.03 (56)	−0.9 (57)	2.5	0.2 (58)	3.2
Methylene Blue	675 [1.8]	0.5 (59)	0.01 (60)	1.8	–	–
Acridine Orange	477 [2.6]	0.5 (61)	−0.9 (62)	1.7	0.4 (63)	2.2
Rose Bengal	567 [2.2]	0.8 (56)	−0.5 (64)	1.7	0.3 (65)	1.9
Hypericin	595 [2.1]	0.7 (52)	−0.6 (66, 67)	1.5	0.9 (66, 67)	1.1
$AlPc(SO_3H)_4$	688 [1.8]	0.4 (52)	−0.3 (68)	1.5	0.9 (69)	0.9
Porphyrazins	650 [1.9]	0.3–0.6 (70)	−0.4 (70)	1.5	0.4 (71)	1.5
Chlorin e_6[‡‡]	665 [1.9]	0.6 (52)	−0.6 (72)	1.3	0.5 (72)	1.4
$Ru(bipy)_3^{2+}$	453 [2.7]	0.7 (73)	−1.6 (74)	1.1	1.0 (24)	1.1
Zinc porphyrin	595 [2.1]	0.9 (52)	−1.8 (53)	0.3	1.1 (75)	1.0

*First excited singlet state energy (in brackets) estimated by the crossing wavelength between the absorbance and fluorescence emission spectra. †$^1O_2$ quantum yield. ‡Pseudoreduction potential of the oxidant photosensitizer. §Pseudoreduction potential of the reductant photosensitizer. ‖Average values of chlorophyll a and b were used. ¶Bacteriochlorophyll data, using the reduction potential value of chlorin e_6. #Hematoporphyrin IX and Protoporphyrin IX have similar values of those added in the table, which are for a free-base porphyrin without substitution. **Free-base porphyrin is not found in the skin of healthy human subjects, but accumulates in the skin of porphyria patients and is present in several bacteria strains, being responsible for the anti-bacterial effects of blue light (76). ††One-electron oxidation potential of porphyrins varies from 1-1.7 depending on the ring substitutions. ‡‡Pheophorbide a and BPD-MA have similar values to those of chlorin e_6.

excited state photosensitizer, that is, the photosensitizer acts as a photochemical reductant (Equation 21). In this case, any photosensitizer that has a one-electron oxidation peak in the voltammogram can potentially be a very strong excited state reducing agent. It is worth mentioning here the case of positively charged photosensitizers such as phenothiazinium salts that do not have an oxidation peak and therefore cannot act as a photochemical reductant.

$$\mathrm{Sens}^* + \mathrm{S} \rightarrow \mathrm{Sens}^{\bullet+} + \mathrm{S}^{\bullet-} \tag{21}$$

Note also that $-E'_{red}$ ($Sens^{\bullet+}/Sens^*$) values are highly favorable (close or above 1V) for most endogenous and exogenous photosensitizers (Table 1). However, there are not many biomolecules that can accept an electron. NAD^+, which is a strong two-electron biological oxidant, has an unfavorable value for one-electron reduction (−0.9, Table 3). O_2 has a reduction potential of −0.3V and is also highly prevalent in many tissues. Therefore, O_2 can potentially receive an electron from most photosensitizers forming $O_2^{\bullet-}$ (Reaction 2). However, as mentioned before, the formation of $O_2^{\bullet-}$ by photosensitizer oxidation is not the most prevalent interaction between the sensitizer and O_2. Intersystem crossing of the photosensitizer to the triplet excited state followed by energy transfer to O_2 to form $^1O_2$ is usually much more probable.

In the case of PCET (see Section 3), in general terms, this process combines redox with acid–base equilibria and the energy necessary for breaking a X–H bond (C–H, O–H, N–H) is given by the homolytic bond-dissociation free energy (BDFE), which can be estimated by using Equation 22 (99).

$$\mathrm{BDFE}_{(X-H)} = 1.4\mathrm{p}K_A + 23.1E^\circ + C_G \tag{22}$$

The constant C_G includes the $H^+/H^\bullet$ standard reduction potential and the formation free energy of $H^\bullet$ in a specific solvent. The value of C_G in water (for NHE) is 57.6 kJ mol^{-1}. As indicated in equation 5, changes in the reduction potential (E^o) can be counter-balanced by changes in pK_A and vice versa. Overall, acidic or conjugate acid species are stronger oxidants. In order to evaluate the possibility that an excited state can break a specific X–H bond, one can use in equation 5 the pseudoreduction potentials from Table 1. Likewise, in order to evaluate the strength of an X-bond in a biological substrate, one could use the reduction potential values from Table 2. The abstraction of hydrogen from biological targets with allylic/bis-allylic hydrogens or with hydroperoxyl radicals in membranes is fundamental to the initiation and the progress of the lipid peroxidation reactions (100). Indeed, recent evidence indicates that type I photosensitized oxidation reactions, involving HAT both from the original lipid double bond or from the lipid hydroperoxides, are necessary and sufficient to form lipid truncated aldehydes, which are the molecules that facilitate membrane leakage (101). Likewise, the abstraction of hydrogen from amino acids (tyrosine, for example) or nitrogen heterocycle bases (guanine, for example) is

Table 2. Reduction potential of biological targets

One-electron reduction	$E^{\circ\prime}$ (V)
α-TO, H^+/α-TOH	0.5 (87)
$PUFA^\bullet$, H^+/ PUFA–H	0.6 (88)
$H\text{-}Asc^\bullet$, H^+/$H\text{-}Asc^-$	0.7 (89)
$RS^\bullet/RS^-$ (Cys)	0.9 (90)
$Allyl^\bullet$, H^+/allyl–H	1.0 (88)
$Trp^\bullet$, H^+/TrpH	1.0 (91)
$TyrO^\bullet$, H^+/TyrOH	1.0 (91)
$ROO^\bullet$, H^+/ROOH	1.0 (88)
$RO^\bullet$, H^+/ROH	1.6 (88)
$dG^{\bullet+}/dG$	1.5 (92)
$dA^{\bullet+}/dA$	1.9 (92)
$dT^{\bullet+}/dT$	2.1 (92)
$dC^{\bullet+}/dC$	2.1 (92)

Table 3. Reduction potential and reactivity of oxidant species.*

	Reduction potential ($E^{\circ\prime}$, V) (88,89,93,94)	k_{GSH}† (M^{-1} s^{-1}) (95–97)
One electron		
$HO^\bullet$, H^+/H_2O	2.3	1×10^{10}
$CO_3^{\bullet-}$, H^+/HCO_3^-	1.8	5×10^7
$O_3^{\bullet-}$, $2H^+/H_2O$, O_2	1.8	7×10^7
$NO_2^\bullet/NO_2^-$	1.0	3×10^7
$HO_2^{\bullet-}$, H^+/H_2O_2	1.1	4×10^5
$O_2^{\bullet-}$, $2H^+/H_2O_2$	0.9	~10 to 10^3
O_2 ($^1\Delta_g$)/$O_2^{\bullet-}$	0.7	2.4×10^6
$O_2/O_2^{\bullet-}$	–0.3	–
$NAD^+/NAD^\bullet$	–0.9	–
Two electron		
H_2O_2, $2H^+/2H_2O$	1.7	0.9
ONOOH, H^+/NO_2^-, H_2O	1.4	7×10^2
HOCl, H^+/Cl^-, H_2O	1.3	3×10^7
O_2, $2H^+/H_2O_2$	0.3	–

*Table modified from (98). †Reactivity against GSH.

fundamental to the understanding of the consequences of the photosensitized oxidations and the autoxidation process in cells and tissues (102).

Many other reactive oxidants (RO) can be formed as the result of the redox reactions initiated by type I photosensitization. It is worth mentioning that several different definitions are currently used for ROS, which makes that the species included in this group differs for different authors. The term RO is broader than ROS and includes any chemical entity able to oxidize biomolecules. In Table 3, we mention the most important oxidizing radicals and two-electron oxidants, with their respective reduction potentials. We also include information of the reactivity toward glutathione, which is an important player in the maintenance of the redox homeostasis. Note that several RO are strong oxidants ($E^{\circ\prime}$ above 1 V), with second-order rate constant in the reaction with glutathione above 10^7 M^{-1} s^{-1} ($HO^\bullet$, $CO_3^{\bullet-}$, $O_3^{\bullet-}$, $NO_2^\bullet$ and HOCl). Others like $O_2^{\bullet-}$ and H_2O_2 are not so reactive, but exert fundamental role in redox signaling and their accumulation invariably will lead to the formation of other RO like $HO^\bullet$ (103). It should be noted that the pseudoreduction potential of several photochemical oxidants is as high as that of $HO^\bullet$ (Table 1). Therefore, it is important to consider that an abundant number of photosensitized oxidant events will be driven by the photosensitizers working as photochemical oxidants.

One-electron oxidation of nucleobases

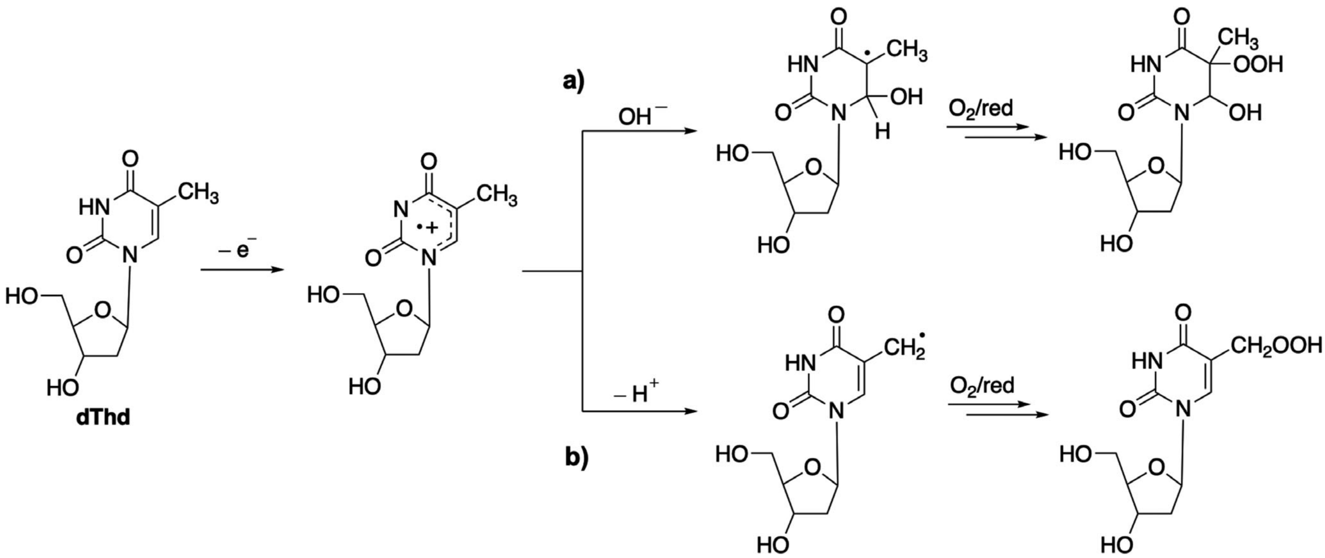
The reactivity of the nucleobases in type I photosensitized one-electron oxidation is modulated in double-stranded DNA by the occurrence of efficient charge transfer reaction that leads through hopping mechanisms to the redistribution of initially generated base radical cations with preferential trapping of positive holes at guanine sites in a highly sequence dependence manner (85). Similarly, type I reactions are facilitated by electron-transfer photooxidations with the use of electron-deficient sensitizers, such as *N*-methylquinolinium tetrafluoroborate, 10-methylacridine hexafluorophosphate or 2-(4-methoxyphenyl)-4,6-diphenylpyrylium in their photosensitized reactions with sulfides (104–107).

The base cation ($S^{\bullet+}$) that is issued from one-electron oxidation of the target is expected to undergo two competitive reactions (hydration, deprotonation) in aqueous solutions that represent suitable conditions for mimicking reactivity and subsequent chemical reactions of oxidizing radicals in cells (108,109). Both reactions give rise to neutral radicals that may be ranged into several distinct reactive intermediates with different chemical reactivity according to the target molecule. The main processes for nucleobases and amino acids can be summed up as follows:

1 Hydration of pyrimidine $S^{\bullet+}$ gives rise to C-centered radicals (Scheme 3a) that efficiently react with O_2 to produce peroxyl radicals; these transient species may be either reduced, likely by $O_2^{\bullet-}$, into related hydroperoxides or react selectively with vicinal bases/sugar moiety in DNA, thus forming intrastrand tandem lesions (110,111).
2 Hydration of $S^{\bullet+}$ derived from purine bases generates aminyl type radicals that are not prone to O_2 addition (112,113). However, 8-hydroxy-7,8-dihydroguan-7-yl radical, the hydration product of guanine radical cation reaction (Scheme 4a) may be converted into 8-oxo-7,8-dihydroguanine (8-oxoG) by O_2-mediated one-electron oxidation (114). Competitive one-electron reduction of the latter radical that already occurs in aqueous solution is enhanced in cells by the presence of thiol components. This leads to the formation of 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG), a nonoxidation modified product of guanine (114,115) (Scheme 4a).
3 Deprotonation of thymine and 5-methylcytosine radical cations produces C-carbon centered radicals (Scheme 3b) to which O_2 efficiently adds to generate peroxyl radicals as observed for the hydration reactions of pyrimidine base radical cations (109).
4 Deprotonation of $S^{\bullet+}$ derived from guanine, tyrosine and tryptophan gives rise to highly oxidizing $S(\text{-}H)^\bullet$ radicals that do not react with O_2 but with $O_2^{\bullet-}$ to produce identified oxidation products. A complex multi-step pathway subsequent to $O_2^{\bullet-}$ addition at C5 of $G(\text{-}H)^\bullet$ has been proposed for the formation of 2,2,4-triamino-5(2*H*)-oxazolone, the final one-electron oxidation guanine product (114, 116) (Scheme 5). Evidence has been provide for chemical repair of $G(\text{-}H)^\bullet$ by $HO_2^\bullet/O_2^{\bullet-}$ and also by thiols.

DNA-protein cross-links

Another interesting example of subsequent reactions in type I mechanisms is the formation of DNA-protein cross-links (DPCs), a wide variety of biomolecular damage that may be generated by various chemical and physical agents including *bis*-electrophilic



Scheme 3. Hydration and deprotonation of thymidine radical cations giving rise to hydroperoxides via transient peroxyl radicals.

agents, low-intensity UVC light and ionizing radiation (117). Thus, high-intensity UV laser pulses (118,119) and several type I photosensitizers (such as methylene blue and riboflavin) that are able to ionize nucleobases were shown to induce the formation of protein/amino acid adducts mostly to the guanine moiety of either nucleic acids or model compounds (120–123). The free Ɛ-amino group of central lysine in trilysine peptide (KKK) bound to TGT was found to compete efficiently with surrounding water molecules for the nucleophilic reactions underwent by $G^{\bullet+}$, generated by riboflavin photosensitization in aerated aqueous solution (124). The major photoproduct that was isolated by HPLC and unambiguously characterized by ^{1}H NMR and exact mass spectrometry measurement was assigned as N^{ε}-(guanin-8-yl)-lysine adduct arising from the addition of the lysine residue to C8 of guanine, similarly to the hydration of $G^{\bullet+}$ (Scheme 4b). A recent *ab initio* molecular dynamics simulation study with protonated methylamine as the model amino acid has provided further mechanistic information on the formation of the guanine-lysine cross-link (125). It is proposed that initial deprotonation of $G^{\bullet+}$ is followed by hydrogen transfer from the ammonium $-NH_3^+$ to $G(-H)^{\bullet}$ with subsequent regeneration of guanine by chemical repair. Concomitantly, this leads to the formation of a nitrogen centered radical that reacts with guanine by addition at C8. Similar cross-links were found to be generated by nucleophilic attachment of three polyamines including putrescine, spermine and spermidine to DNA at C8 of $G^{\bullet+}$ upon riboflavin photosensitization (126,127). Furthermore, advanced glycation end products were reported to function as sensitizers and induce oxidation and crosslinking of bovine lens proteins by mainly a type I reaction (128).

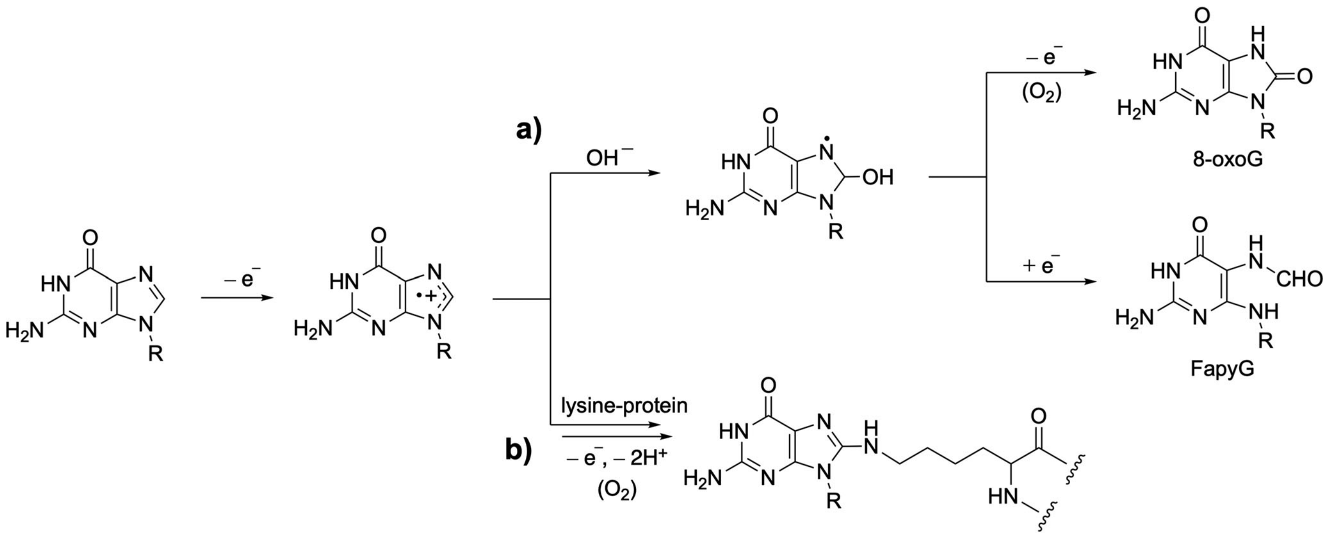
One-electron oxidation of amino acids

Several amino acids can undergo reaction 1 and the resulting radicals $S^{\bullet+}/S(-H)^{\bullet}$ participate in many different subsequent processes. Studies performed with Trp and Tyr free and in proteins allow describing a general behavior. Radicals can react with O_2 to generate peroxyl radicals as precursors of multiple oxygenated products including hydroperoxides and carbonyls (129,130). These reactions compete with self-reactions of radicals, which lead to intra- or intermolecular crosslinks (131) (*vide supra*). Several studies using riboflavin as photosensitizer have evidenced the competition between these pathways (7,31,132). Under aerobic conditions the O_2 concentration is much higher than the concentration of the radicals generated by type I photosensitizers. However, self-reactions of radicals are relevant and kinetic analysis provides an explanation for this fact. While the bimolecular rate constant of the reaction of Trp and Tyr radicals with O_2 are low ($k < 10^{6}$ M^{-1} s^{-1}) (133,134), the reactions between radicals are close to the diffusional limit ($k \sim 10^{8}$–10^{9} M^{-1} s^{-1}) (135,136). Many studies with other sensitizers have provided evidence for the complexity of the reactions, in which many factors affect the distribution of products and the overall competition of type I and type II mechanisms (6,137–141).

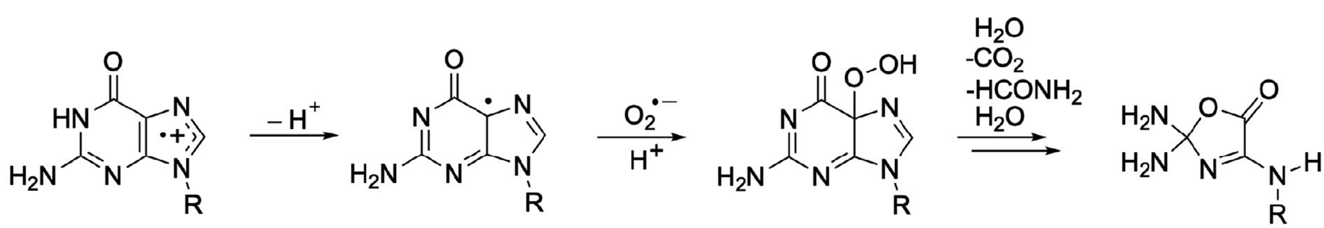
One-electron oxidation of lipids

Photoinduced lipid oxidation has similarities and differences compared with chemically initiated lipid oxidation. The main similarity relates with the self-sustained continuation of its free-radical chain reactions. Several reactive free-radicals, such as carbon-centered, peroxyl, alkoxyl, can react with oxygen and/or abstract hydrogens from allylic hydrogens to continue the degradation of the biomembranes. The main difference concerns the initiation step (100, 101). Purely chemical initiation is highly dependent on bis-allylic hydrogens present in poly-unsaturated fatty acids (PUFA), since the reduction potential of PUFA lipids (~0.6 V) is greatly decreased compared with the reduction potential of lipids with a single double bond (~1 V, Table 2). Photoinduced lipid oxidation does not depend on the presence of PUFA lipids, since both type I and type II reactions can be highly efficient in single saturation lipids. Most photosensitizers have pseudoreduction potentials above 1 V, allowing the oxidation of single double bonds (Table 1). No wonder that light-induced oxidation is one of the major factors responsible for food waste (142).

Type I photosensitization of phospholipids is complex and involves a large number of competitive pathways giving rise to



Scheme 4. Type I photosensitized reaction of guanine. Nucleophilic reactions of the guanine radical cation. Formation of (a) 8-oxo-7,8-dihydroguanine (8-oxoG) and 2,6-diamino-4-hydroxy-5-formamidopyrimdine (FapyG) via hydration and (b) lysine-guanine addition product.



Scheme 5. Type I photosensitized reaction of guanine in a multistep formation of 2,2,4-triamino-5(2*H*)-oxazolone via $O_2^{\bullet -}$ addition to the deprotonated guanine radical cation.

many photoproducts, whose distribution depends on many factors, starting with the nature of the reactant. However, as mentioned in the previous paragraph, the first event is always the abstraction of an allylic hydrogen from the unsaturated fatty acyl group. This reaction in PUFAs leads to a radical ($L^{\bullet}$), with its free electron delocalized over five carbons, that reacts with O_2 to give a peroxyl radical ($LOO^{\bullet}$) (143). Scheme 6 shows the reactions that take place for a typical glycerophospholipid (LH) bearing a linoleoyl group (18:2-9,12). $L^{\bullet}$ reacts with O_2 preferentially at positions 9 and 13 to form the corresponding $LOO^{\bullet}$ radicals. In the propagation phase, the subsequent reaction of these radicals with LH generates the 9- and 13-hydroperoxides and new $L^{\bullet}$ that will react with O_2. The chain reactions are limited by the availability of O_2 and oxidizable lipids, and the presence of antioxidants that can donate a hydrogen atom to $LOO^{\bullet}$ (143). It is worth mentioning, that we have respected the most common nomenclature found in literature for lipids, where LH represents the intact substrate (S) and $L^{\bullet}$ is the radical resulting from the loss of an hydrogen atom ($S(-H)^{\bullet}$).

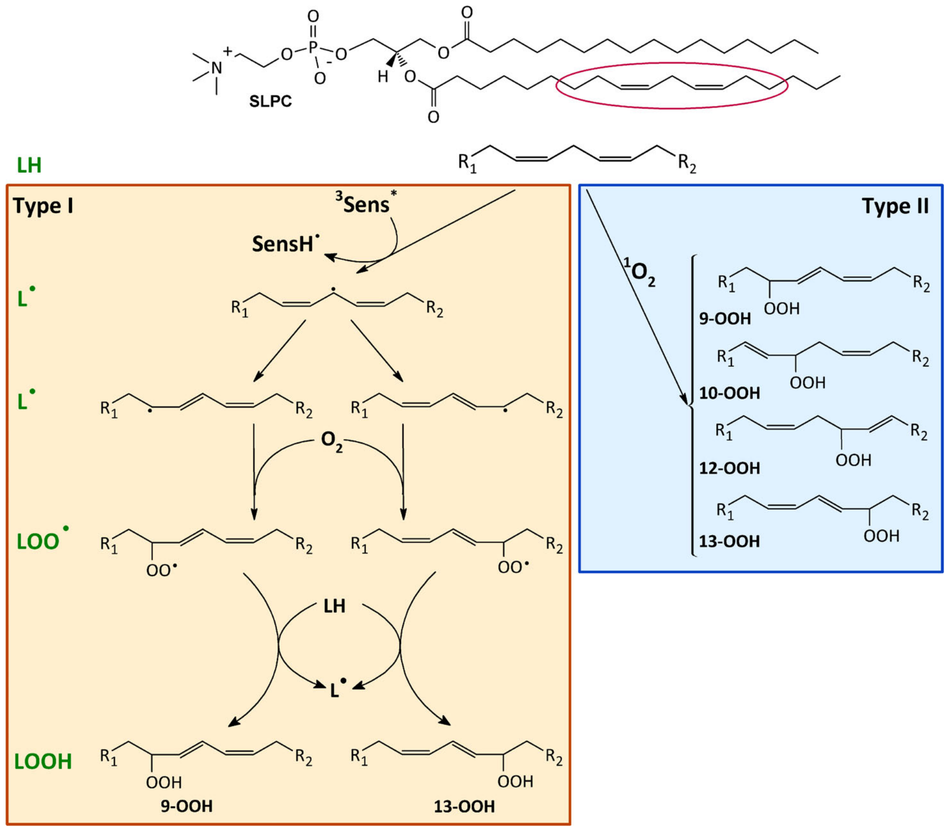
TYPE II PHOTOSENSITIZED OXIDATION

General features

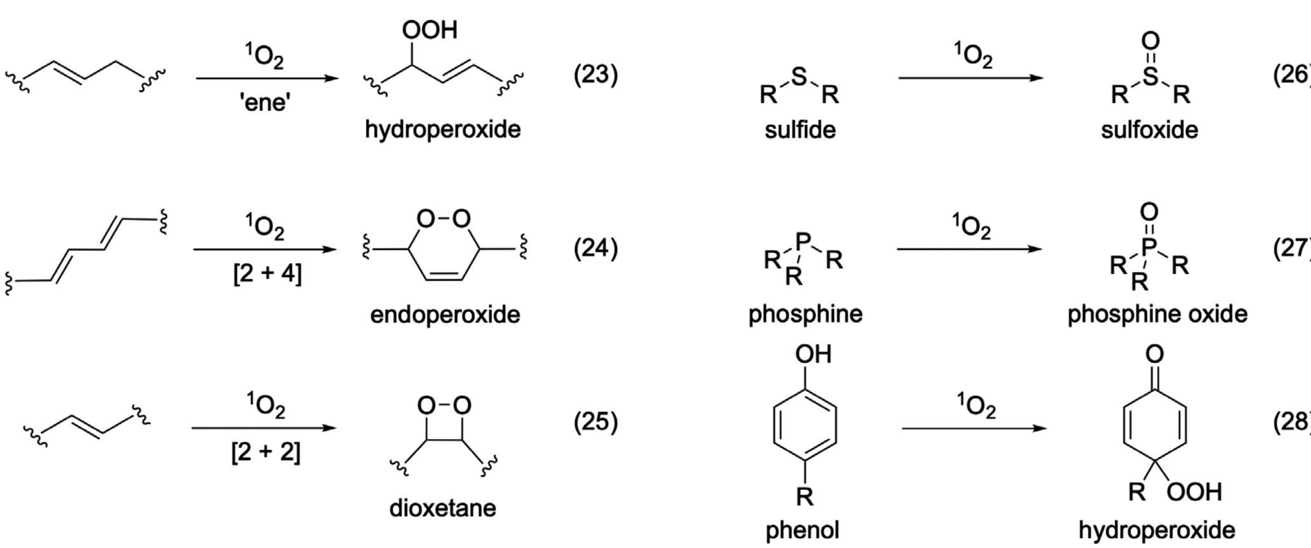
Type II photosensitized oxidations involve $^1O_2$, generated in reaction 3 that reacts with biomolecules. Singlet oxygen is a more selective oxidant than $HO^{\bullet}$ and one-electron oxidants. Reactions of unsaturated compounds with $^1O_2$ include "ene" (reaction 23), [2 + 4] (reaction 24), and [2 + 2] (reaction 25) reactions to yield hydroperoxides, endoperoxides and dioxetanes, respectively (Scheme 7). Other relevant reactions of $^1O_2$ include heteroatom oxidation (reactions 26 and 27) and phenol hydroperoxidation (reaction 28) (Scheme 7) (18,22,144). Singlet oxygen is also important in inflammation processes (145).

Singlet oxygen oxidation of nucleic acids

Only guanine among the 5 main canonical pyrimidine and purine DNA bases exhibits a detectable reactivity toward $^1O_2$ in aqueous solutions as shown from extensive chemical studies (18,146,147). The selective $^1O_2$ reactivity that is in agreement with the highest chemical quenching rates of guanine components has recently received further support from the conclusions of theoretical studies (148,149). Early evidence has shown that 8-oxoG, a ubiquitous oxidation product of guanine (114), was generated in isolated DNA by thiazin dyes that mostly act as type II photosensitizers (150–152). It was confirmed by using thermo-labile naphthalene endoperoxides as clean sources of $^1O_2$ that 8-oxoG is the only $^1O_2$ degradation product formed in isolated DNA under mild oxidation conditions (147) in agreement with recent reactivity studies involving molecular dynamics simulation (148). The formation of 8-oxoG that was found to be almost barrierless (148) is rationalized in terms of [2 + 4] Diels-Alder cycloaddition of $^1O_2$ across the 4,5- and 7,8-ethylenic

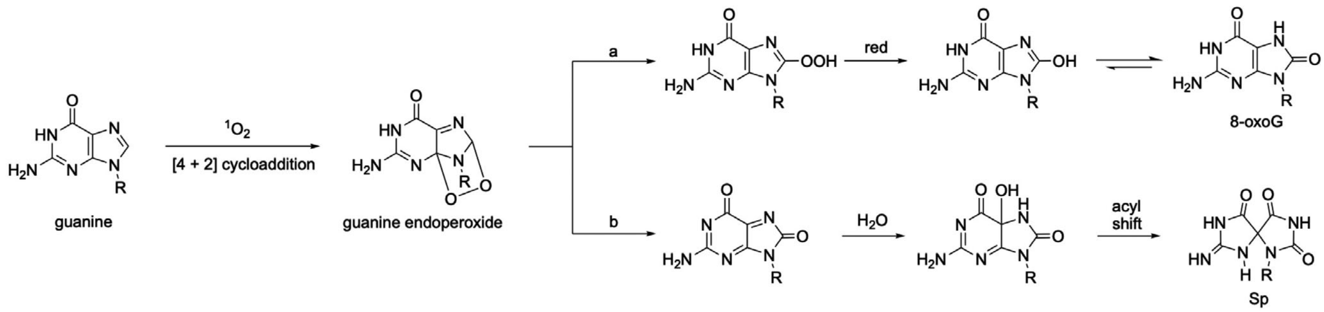


Scheme 6. Type I and type II photosensitized lipid peroxidation. PLPC, 1-Palmitoyl-2-linoleoyl-sn-glycero-3-phosphatidylcholine, is an example of a phospholipid containing a saturated fatty acid and a polyunsaturated (PUFA) fatty acid, where the photoinduced peroxidation takes place.

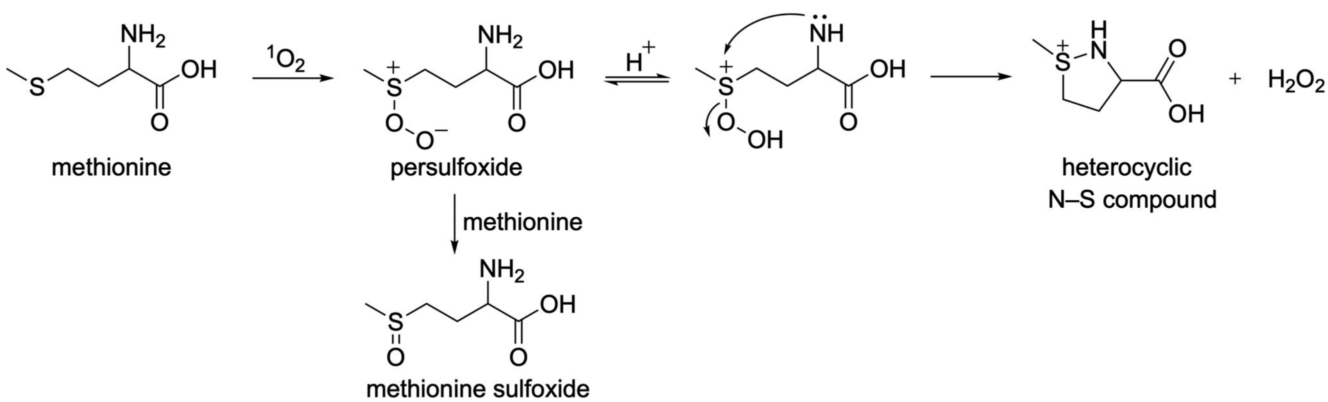


Scheme 7. Common reactions of $^1O_2$ with organic compounds.

bonds of the imidazole ring to give rise to a 4,8-endoperoxide (114) (Scheme 8) that has been only characterized in CD_2Cl_2 solutions of photosensitized 2',3',5'-*O*-(*tert*-butyldimethylsilyl)-8-methylguanosine at –78 °C (153). Further mechanistic information of the $^1O_2$ oxidation pathway of guanine in either isolated 2'-deoxyguanosine or embedded into a double-stranded DNA fragment was gained from extensive theoretical studies. Thus, nucleophilic attack of $^1O_2$ onto guanine C8 gives rise to the 4,8-endoperoxide via a zwitterionic peroxylate (-OO$^-$) according to a two-step pathway (148). The exclusive formation of 8-oxoG in DNA is rationalized in terms of predominant rearrangement of the endoperoxide into 8-hydroperoxyguanine (154) as further supported by a combination of DFT and *ab initio* computational studies (155). This is followed by the conversion of the unstable



Scheme 8. Singlet oxygen oxidation of guanine in isolated nucleosides (a + b) and DNA (a).



Scheme 9. Reaction of methionine with singlet oxygen in aqueous solution.

peroxide intermediate into 8-hydroxyguanine that is in dynamic equilibrium with 8-oxo-7,8-dihydroguanine (8-oxoG) (Scheme 8 b), the more stable tautomer in solution (114,154). In contrast a more complex oxidation pathway is observed for dGuo and short oligonucleotides with the predominant formation of spiroiminodihydantoin (Sp) over 8-oxoG (156). This is explained by a water assisted rearrangement of the endoperoxide (148) giving rise to a reactive quinonoid (154,157) that via two successive steps including hydration and acyl type rearrangement leads to Sp. The quinonoid has been also shown to efficiently react with amino group of lysine to form guanine-lysine cross-link as substituted Sp derivatives (158). However, both water and lysine addition reactions are abolished in ds-DNA since the endoperoxide rearrangement leading to the quinonoid is kinetically prevented due to a lack of accessibility of reactive intermediates to water molecules (148).

Quenching of triplet-excited 4-thiouracil, a minor component of tRNA, was monitored by ultrafast time resolved spectroscopy (159) as an efficient generator of $^1O_2$ in aqueous solution with a quantum yield of 20% (160). 4-Thiouracil, a strong endogenous UVA sensitizer, is able to efficiently react with $^1O_2$ giving rise to uracil (161) and uracil-6-sulfonate according to oxidative pathways that were elucidated by DFT computations (159).

Singlet oxygen oxidation of amino acids

Mechanistic details of the type II ($^1O_2$) oxidation of methionine have been reported (162) (Scheme 9). Methionine sensitized photooxidation likely leads to a persulfoxide intermediate ($R_2S^+OO^-$). This persulfoxide is zwitterionic, where a reaction with a second methionine leads to two moles of methionine sulfoxide. Whether a similar reaction between a methionine persulfoxide site and a second methionine site in proteins is uncertain, due to potential steric isolation (163). The reaction of methionine with $^1O_2$ in solution at pH $\leq$ 6 leads to a single product, methionine sulfoxide. However, at pH 6-10, a heterocyclic N–S compound (dehydromethionine) forms, which hydrolyzes to methionine sulfoxide with formation of H_2O_2 as a by-product. In addition to methionine, organic sulfides exhibit detectable reactivity with $^1O_2$ as has been reported in extensive studies (164,165). Sulfides show ~100% chemical reactivity with $^1O_2$ in protic solvents, but only ~5% in aprotic solvents. In the latter case, physical quenching of $^1O_2$ leading to $^3O_2$ is the main path (~95%). The reason for the efficient reaction in protic media is due to conversion of the persulfoxide to a hydroperoxy sulfurane [$R_2S(OH)OOR'$] via the addition of the OH group from water or methanol. A 1996 report (166) proposed a mechanism involving $^1O_2$ and formation of a persulfoxide followed by reaction with methanol to give a hydroperoxy-methoxy sulfurane, which is consistent with the results. In methanol, only a single intermediate was proposed and suggested to be either a hydrogen-bonded persulfoxide or a hydroperoxysulfurane. The chemical quenching rate constants (k_r) increase by an order of magnitude upon addition of as little as 1.5% methanol in benzene solvent. This large rate enhancement is attributed to a mechanism, which circumvents the energetically costly interconversion of the persulfoxide. In other reactions, mainly in aprotic solvents, evidence points to the intermediacy of thiadioxirane (cyclic-R_2SO_2) and hydroperoxy sulfonium ylides [$R(R'CH-)S^+OO^-$]. Computational evidence has also been reported for these sulfur peroxy intermediates (167).

Singlet oxygen reacts with other amino acids, including Trp, His, Cys and Tyr (168–170). Methionine has been reported to undergo type I and type II reactions based on the reaction conditions (171). For example, the formation of the endogenous photosensitizer, 3-hydroxykynurenine (from Trp), leads to methionine sulfoxide (from Met) and DOPA (from Tyr), mainly by the type I reaction because Φ_Δ values were relatively low (<20%) (172). On the other hand, a di-cyan-hemin sensitized reaction showed evidence for a type II process in the conversion of methionine to methionine sulfoxide, in contrast to cysteine and tryptophan oxidation reactions that involve mixed type I and type II reactions (139,173).

Singlet oxygen oxidation of lipids

Singlet oxygen can react directly with unsaturated fatty acids to yield hydroperoxides with double bonds shifted to the allylic position. This process is an "ene" reaction (Reaction 23, Scheme 6) and with no intervention of free radical intermediates (143). Although the "ene" reaction is faster for PUFA lipids, it occurs for both allylic and bis-allylic hydrogens (174). In contrast to type I photosensitization, the oxidation of PUFAs by $^1O_2$ gives rise to four hydroperoxide isomers. For example, in the case of the linoleoyl group, besides the 9 and 13 hydroperoxides, the 10 and 12 isomers are also formed (Scheme 6).

PHOTOSENSITIZED CYCLOADDITION

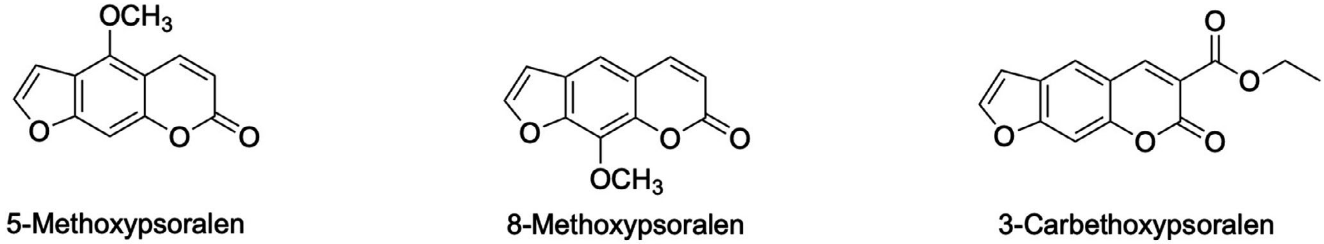
In a photosensitized cycloaddition, the photosensitizer reacts with the substrate, and two covalent bonds are formed between the two molecules, giving rise to a cyclic product. Although different types of photocycloaddition have been described, in biological photosensitization more relevant is the [2 + 2] photocycloaddition, in which the excited photosensitizer with a double bond reacts with a substrate bearing a double bond, to form a product with a cyclobutane cycle.

Mono- and intrastrand psoralen-DNA photoadducts

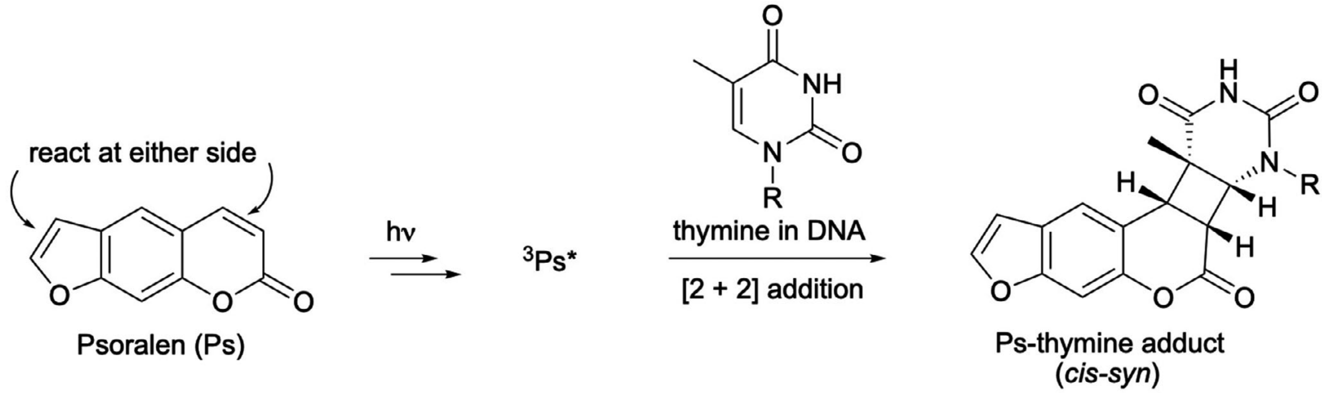
Natural and synthetic bi-functional psoralens and other furocoumarins including monofunctional psoralens (Scheme 10) and angular angelicins are potent UVA sensitizing agents used for PUVA (psoralen + UVA) photochemotherapy of several skin diseases including psoriasis, vitiligo and mycosis fungoides (175–177). Extracorporeal photophoresis is another relevant clinical application of 8-methoxypsoralen (8-MOP) for the phototreatment of cutaneous T-cell lymphoma, scleroderma and organ rejection (178,179). Major information on the photoreactivity of psoralens toward nucleic acids was gained from model studies more than 50 years ago (180). Early evidence was provided for efficient [2 + 2] photocycloaddition of UVA excited psoralens through either the 3,4-ethylenic bond of the pyrone ring or the 4',5'-double bond of the furan moiety to the 5,6-ethylenic bond of thymine (Scheme 11) and to a lesser extent of cytosine (181–184).

This is in agreement with a specific binding of most psoralens including 8-MOP to 5'-TpA (3'side) and 5'-ApT (5'-side) sequences of DNA as inferred from the predominant formation of thymine-8-MOP-thymine diadducts at 5'-TpA cross-linkage sites of DNA (185) and the gel sequencing distribution of 8-MOP-thymine photocycloadducts in DNA fragments (186). UVA excitation of intercalated furocoumarins in native DNA gives rise predominantly as primary photoproducts to pairs of *cis-syn* diastereomers of furan-side monoadducts to thymidine that were carefully characterized by extensive NMR analyses and other spectroscopic measurements for 4'-hydroxymethyl-4,5',8-trimethylpsoralen (HMT) (187), 8-MOP, 4,5',8-trimethylpsoralen (TMP) (188) and 5-methoxypsoralen (5-MOP) (189). Subsequent absorption of an UVA photon by furan-side mono monoadducts to thymidine leads to the efficient formation of interstrand cross-link as single pairs of *cis-syn* diastereomers (190). The photocycloaddition of the coumarin moiety of furan moiety of 8-MOP to the thymidine on the opposite side is an efficient photoreaction that occurs with a 4-fold higher quantum yield than that of initial formation of the monoadduct (185). It was also found that UV irradiation of furan-side HMT-thymidine monoadduct wavelength above 313 nm lead to quantitative photo-cross-linking whereas competitive photoreversion is observed at shorter wavelengths (191). Evidence was also provided for the UVA-induced formation of DNA-8-MOP-protein cross-link through the reaction of furan-side monoadduct with amino acid (192). Pyrone-side monoadducts of bifunctional psoralens to thymidine do not absorb in the UVA range and therefore are unable to be converted into cross-links (187,188). The low formation efficiency together with the instability of the pyrone-side monoadducts explain the difficulty to unambiguously assign the stereoconfiguration of most thymidine adducts at the exception of those of 5-MOP that were identified as two *cis-syn* diastereomers (193). 3-Carbethoxypsoralen (3-CPs) and 7-methylpyrido[3,4-c]psoralen (MePyPs) for which the pyrone moiety exhibits either a bulky substituent or a fused pyridine ring are only able to react with pyrimidine bases through the photoreactive 4',5'-furan ethylenic bond. Thus, the two *cis-syn* diastereomers of furan-side monoadducts of either 3-CPs (194–196) or 7-methyl-pyrido[3,4-c] psoralen (MePyPs) (197) to thymidine have been fully characterized. 3-CPs furan-side monoadducts to 2'-deoxycytidine represent only 1% of the pyrimidine adducts, thus suggesting that intercalation of 3-CPs takes place preferentially at AT sites as previously observed for bifunctional furocoumarins.

The quantum yield and reactivity of the UVA triplet-excited furocoumarins including 8-MOP, 5-MOP, TMP, 3-CPs (198–200) and furan-side thymidine monoadducts (201) were determined by laser flash photolysis. However, no transient was observed upon intercalation of the psoralens into DNA (199,202). This was recently explained by an efficient photoinduced electron transfer reaction from guanine to intercalated AMT inside either DNA (203,204) or human telomeric G-quadruplex (205) as shown by femtosecond transient absorption spectroscopy. Further information on excited psoralen transients, photobinding of bifunctional furocoumarins to thymine and the reactivity of the resulting furan- and pyrone-side monoadducts was gained from quantum chemistry studies (206–208). The inability for pyrone-side adduct of 8-MOP to thymine to be converted into interstrand cross-links was explained by the blueshift of their S_1 excitation energy with respect to isolated photosensitizer, thus preventing any further UVA-mediated reaction (207,208). It was shown from ONIOM and hybrid DFT calculations that the formation of furan- and pyrone-side monoadducts to thymine involves a psoralens triplet excited state as the precursor of a biradical. In subsequent steps, a covalent bond is formed between either the furan or the pyrone moiety and C6 thymine before cyclobutane ring closure (209). Similar



Scheme 10. Psoralen structures. Mono (3-carbethoxypsoralen) and bifunctional (5-methoxypsoralen, 8-methoxypsoralen) psoralens.



Scheme 11. Psoralen photocycloaddition reactions. Formation of *cis-syn* pyrone-side monoadduct to thymine.

conclusions were reached for AMT, 8-MOP, 5-MOP and TMP from detailed studies including nanosecond UV-vis and IR absorption spectroscopy together with IR computation (210,211).

TRIPLET–TRIPLET ENERGY TRANSFER

Two main types of triple–triplet energy transfer (TTET)-mediated photosensitization reactions of nucleic acids and their pyrimidines bases have been observed. Cyclobutane pyrimidine dimers (CPDs) with predominance of cyclobutane thymine dimers (T<>T) are formed in aqueous solutions via [2 + 2] cycloaddition of an excited thymine to a vicinal pyrimidine base in the ground state. A different structural isomer of T<>T and also TT pyrimidine (6-4) pyrimidone photoproduct ((6-4)PP) that was characterized as (5*R*)-5-(thyminyl)-5,6-dihydrothymine (212), the so-called "spore photoproduct" (SP) is generated in either frozen aqueous solutions or the dry state (213). In both cases, the triplet energy (E_T) of the donor has to be higher or at least similar to that of the pyrimidine base for triggering the formation of either CPDs or SP (214). In addition, the efficiency of the intersystem crossing that allows the conversion of the singlet excited state of the photosensitizer into its triplet excited state and the life time of the triplet excited state are also critical parameters.

Sensitized formation of cyclobutane pyrimidine dimers (CPDs)

The first evidence for the photosensitized formation of T<>T in isolated DNA (215) was provided using acetophenone as the triplet photosensitizer. Simultaneously, other UVA-excited ketones including acetone, propriophenone and benzophenone were found to generate cyclobutane pyrimidine dimers for several pyrimidine bases including thymine (216), 1,3-dimethyluracil (217) and orotic acid (218). These early findings have provided a strong impetus to the assessment of additional photosensitizer features (for an earlier comprehensive review, see 219) and further development of mechanistic studied involving relevant biochemical photosensitizers in the last three decades. These include among others pyridopsoralens (220–222), fluoroquinolones (FQ) (223–227) and nonsteroidal ketoprofen derivatives (228). Accurate information on the quantitative distribution of photosensitized generation via TTET mechanism of the three thymine containing-CPDs in isolated DNA is available from quantitative HPLC-MS/MS measurements (223,227). Thus, predominant generation of *cis-syn* T<>T over relatively minor T<>C and C<>T photoproducts is observed upon photosensitization by either ketones or fluoroquinolones, with lomefloxacin and norfloxacin being the most efficient. It was confirmed using an optimized HPLC-MS/MS method that C<>C are generated in very low amounts (227) in agreement with the higher energy triplet of cytosine with respect to thymine by about 20 kJ mol^{-1}. Interestingly, the comparison of the efficiency for 5 selected FQ with different triplet energies to generate T<>T has led to an estimation of the triplet energy of thymine in double-stranded DNA that is close to 270 kJ mol^{-1} (224,225). This is about 30 kJ mol^{-1} lower than the E_T value for isolated thymine or thymidine 5'-monophosphate.

Internal DNA photosensitizers as CPD generators

Several photoinduced and oxidatively generated base lesions have been identified as potential intrinsic sensitizers to UVA radiation of cyclobutane thymine dimers (T<>T) in isolated DNA and thymine model compounds.

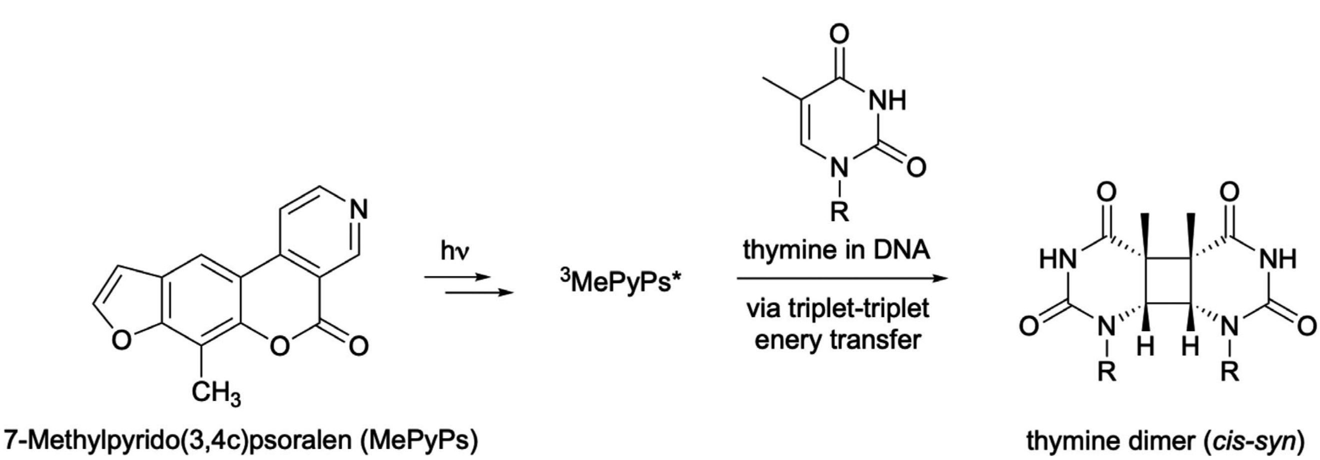
As the first evidence of such photosensitized reactions, it was shown that a furan-side 7-methyl-pyrido(3,4)psoralen (MePyPso) monoadduct to thymine generated in a double-stranded EcoR1-HindIII DNA fragments was able to induce the formation of T<>T in its close vicinity upon subsequent UVA irradiation (229). The efficient sequencing mapping of psoralen adduct and T<>T that was applied provided support for an efficient thymine dimerization at tetranucleotide 5′-TATT-′′ sequence. This complements previous studies showing that isolated mono-functional pyridopsoralens including MePyPso were able to photosensitize pyrimidine base dimerization via the TTET mechanism at 5′-AT-3′ sites where they preferentially intercalate (221,222) (Scheme 12).

Pyrimidine (6-4) pyrimidone photoproducts (6-4PPs), the second major class of UVB bipyrimidine DNA photoproducts, have the potentiality for triggering the photosensitized formation of CPDs. The triplet state energy of 5-methyl-2-pyrimidone (291 kJ mol^{-1}) a model compound that mimics the UVB/UVA absorbance and photophysics features of the pyrimidone (Pyo) moiety of 6-4PPs is higher than that of thymine (267 kJ mol^{-1}) as inferred from the low temperature phosphorescence spectrum (230). It was also shown that the presence of free Pyo (Scheme 13) in UVA-irradiated aqueous solutions of plasmid pBR322 gave rise to the formation of CPDs revealed as T4 endonuclease V-sensitive sites (230) Further insights into the photophysics of the embedded Pyo unit in the ds DNA were gained from detailed molecular-dynamics and DFT simulations, thus confirming occurrence of Dexter-type TTET photosensitization mechanism (231). Similar findings on the photosensitizing potential of relevant thymine 6-4PP that exhibits a 5-hydroxy-5,6-dihydrothymine substituent were provided through a subsequent study involving determination of photophysical features and CPD measurements (232). However, the efficiency of proposed Trojan horse role that could play 6-4 PP in promoting pyrimidine base dimerization has been recently questioned (233). Thus, the UVA sensitized formation of CPDs was not detectable in double stranded DNA in which 6-4PPs were generated by UVB irradiation. This is explained by predominant isomerization of 6-4 PPs into related Dewar valence isomers that was previously shown to be a major modulation reaction of UVA component on initially UVB generated DNA damage upon exposure to solar radiation (234,235).

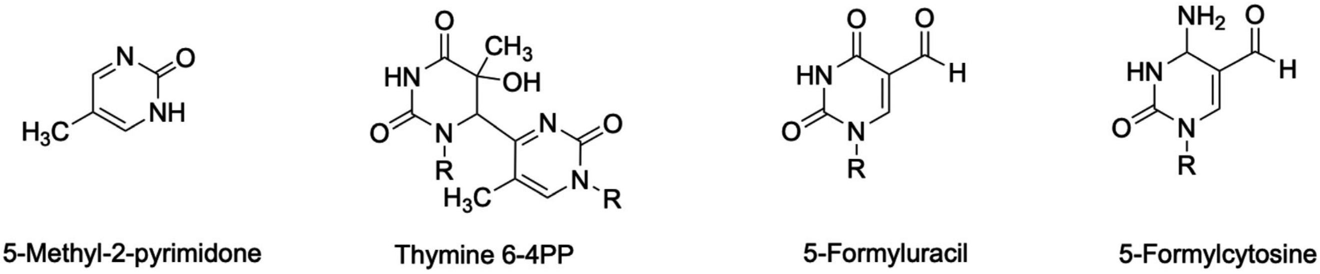
5-Formyluracil (5-forU) (Scheme 13), one of the main oxidatively generated products of thymine by either hydroxyl radical ($HO^{\bullet}$) or one-electron oxidants in isolated and cellular DNA (109), has been found to UVA-sensitize the formation of CPDs in aqueous solutions of pyrimidine-dyads and plasmid DNA (236). This was rationalized in terms of efficient population of $^3\pi\pi^*$ triplet state of thymine via TTET from $^3\pi\pi^*$ excited 5-ForU (E_T = 314 kJ mol^{-1}) as supported by high-level modeling and simulations (237). Evidence was provided for thymine photodimerization when 5-forU is embedded into a ds-DNA structure. Similarly, 5-formylcytosine (5-ForC) that can be generated in cellular DNA by radical oxidation reactions (238) and also enzymatically as an epigenetic mark by ten-eleven translocation dioxygenases (109) exhibits the ability to photosensitize the formation of CPDs, although less efficiently than 5-ForU (239,240). The absorbance of 5-forC that essentially concerns the UVB domain is less redshifted than that of 5-ForU, thus making the oxidized cytosine a rather poor UVA photosensitizer. Furthermore, 5-ForC shows a slower intersystem crossing than 5-ForU. However, it remains to assess the efficiency for either 5-ForU or 5-ForC to trigger the formation of CPDs when the oxidized methyl bases are present in DNA as intrinsic photosensitizers. It may be reminded that the levels of 5-ForU or 5-ForC, both easily repaired base lesions, are rather low reaching a maximum of a few modifications per 10^5 pyrimidine bases in the DNA cells exposed to exogenous oxidants or endogenous enzymatic oxidation. This is likely to affect the efficiency of photosensitized formation of CPDs.

Photosensitized formation of spore photoproduct

It is well documented that UVC irradiation of DNA and free thymidine in the dry state or frozen aqueous favors the formation of the "spore photoproduct" (SP) at the expense of CPDs and 6-4PPs (241–243). Evidence has been provided for the sensitized formation of SP upon UVA-irradiation of thymidine as a film in the presence of either pyridopsoralens (221) or benzophenone (244) that both are efficient TTET photosensitizers. The formation of SP is also predominant over usual bipyrimidine photoproducts in dehydrated spores (245,246). This is explained partly by conformational changes in the DNA structure that result from the desiccation of the dormant spores (247) and the presence of



Scheme 12. Pyridopsoralen sensitized thymine-thymine dimerization.



Scheme 13. Triplet–triplet energy transfer photosensitizers of cyclobutane pyrimidine dimers: photoinduced and oxidatively generated pyrimidine base modifications.

α/ß-type small, acid soluble spore proteins (SASP) (248,249). Interestingly, UVC irradiation of either frozen aqueous solutions of thymidine or dry DNA film upon addition of pyridine-2,6-dicarboxylic acid (dipicolinic acid, DPA), another key spore component led to a significance increase in the SP yield (Scheme 14) with respect to other bipyrimidine photoproducts together with a decrease in the ratio 6-4PPs/CPDs (250). Evidence was also provided for the formation of CPDs at the exclusion of 6-4PP upon exposure of aqueous solutions of thymidine and DPA to UVC radiation (250). These data are strongly suggestive of the implication of efficient photosensitization reactions of pyrimidine bases mediated by DPA via TTET. This has recently received further confirmation from a comprehensive photophysical study of DPA that mostly absorbs in the UVB range with a maximum centered at 300 nm (251). Thus, an efficiently bimolecular quenching rate ($k_q = 5.3 \times 10^9$ M^{-1} s^{-1}) was determined for the reaction of thymidine with DPA in the triplet excited state. In addition, the measured triplet energy (E_{at} = 328 kJ mol^{-1}) for DPA is much higher than that of thymidine thus accrediting the predominance of TTET photosensitized reactions in the overwhelming formation of SP in dry spores (252).

CELLULAR PHOTOSENSITIZATION REACTIONS

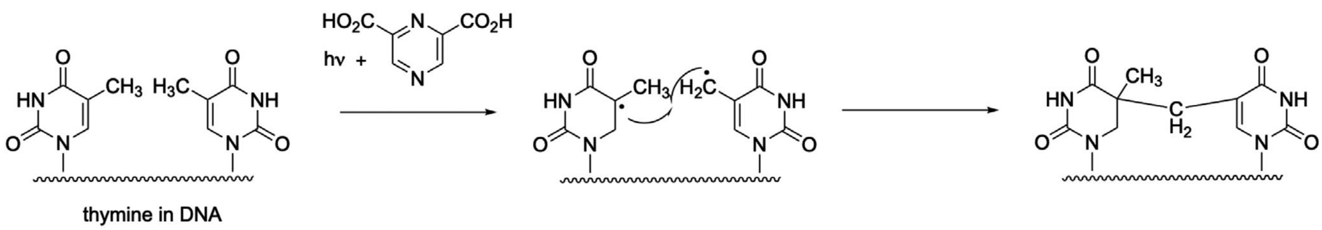
Information on photosensitized formation of damage to nucleic acids, lipids and proteins in cells has been scarce until the development of accurate and sensitive detection methods. This concerns in particular the measurement of oxidatively generated nucleobase modifications that are formed in low yields (at best a few modifications per 10^5 nucleobases) and suffers from several drawbacks according to the assay used. Thus, immunoassays that are relevant for detecting bulky DNA lesions including cyclobutadipyrimidines (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (253) are not suitable for monitoring the formation of oxidized bases, such as 8-oxoG due to cross-reactivity occurrence with overwhelming normal bases (254,255). HPLC-based methods that include either electrochemical detection or tandem mass spectrometry as the detectors have the required sensitivity to measure 8-oxoG the main photosensitized DNA oxidation product in cells and skin (254). However, application of these methods is restricted to heavily oxidized DNA due to occurrence of artefactual oxidation reactions during DNA extraction and subsequent workup before HPLC measurement. Sensitive but less specific detection of low amounts of oxidized purine and pyrimidine bases is achieved using modified versions of either alkaline comet assay (256) or alkaline elution technique (257) that involve a preincubation step with DNA repair *N*-glycosylases to reveal base damage as additional strand breaks.

Type I photosensitized reactions: one-electron oxidation of guanine in cellular DNA

One of the first examples of photosensitized formation of 8-oxoG in cellular has involved riboflavin that predominantly operates through type I photosensitization mechanism (258–260) (Scheme 4a). Visible light irradiation of either mouse lymphoma L5178 (261) or mouse mammary FM3A cells (262) treated with riboflavin led to a significant increase in the level of 8-oxo-7,8-dihydro-2’-deoxyguanosine (8-oxodGuo) over cellular background. Evidence was also provided for a fast repair of photosensitized 8-oxodGuo since 60–7% of the lesions were removed after 2h postincubation (262) what is compatible with implication of the base excision repair pathway. Model studies have shown that type I riboflavin-mediated photosensitization of isolated DNA gave rise to 8-oxodGuo through hydration of the radical cation of guanine and nonsinglet oxidation reaction (263). This recently received confirmation from sequencing DNA mapping experiments showing preferential photosensitized formation of 8-oxodGuo at the 5’-site of GG doublets (264) that exhibits a relatively lower ionization potential and was proposed as an indicator of type I photosensitization mechanism (259,265). It remains to seek whether in cellular DNA FapyG, another main degradation product of guanine radical cation (119,266) (Scheme 4a) is found, an expected base modification of riboflavin type I photosensitized reaction (267).

Type II photosensitized reactions: singlet oxygen oxidation of guanine

Initial studies on photosensitized formation of oxidatively generated damage to cellular DNA have involved the detection of DNA repair enzyme-sensitive sites that were measured as additional DNA strand breaks using the alkaline elution technique (257,268). Thus, visible light excited polar Ro19-8022 ([*R*-1-[(10-chloro-4-oxo-3-phenyl-4*H*-benzo[α]quinolizin-1-yl)-carbonyl]-2-pyrrolidine-methanol induced the predominant formation of formamidopyrimidine DNA *N*-glycosylase (Fpg)-sensitive sites together with a low generation of direct/alkali-labile DNA strand breaks in AS52 Chinese hamster ovary cells (269). Interestingly, a similar DNA oxidation profile was observed upon exposure of AS52 cells to a thermolabile naphthalene endoperoxide of *N,N’*-9-di(2,3-dihydroxypropyl)-1,4-naphthalenedipropanamide ($NPPO_2$), a clean chemical source of



Scheme 14. Sensitized formation of the "spore photoproduct".

$^1O_2$ (270). In addition, direct evidence for the formation of 8-oxoG, one of the preferential DNA substrates of Fpg protein, was provided by HPLC-EC measurements. These observations are fully consistent with a predominant type II mechanism for Ro19-8022 since it was further confirmed that treatment of the human cells with $NPPO_2$ led to the overwhelming formation of 8-oxoG at the exclusion of DNA strand breaks (271,272).

Other type II photosensitizers that can function as solar light sensitive drugs and trigger adverse side effects have received major attention. Several fluoroquinolone antibiotics have been shown to be highly phototoxic and phototumorigenic (273–275) in relation with their UVA-sensitized genotoxic effects (276–279). UVA irradiation of pretreated adult rat liver (ARL18) cells with lomefloxacin and ciprofloxacin was found to lead to a six- and three-fold increase respectively in the cellular level of 8-oxodGuo (280) as mostly the result of type II photosensitization mechanism (Scheme 8a). In a subsequent study, norfloxacin and to lesser extent ofloxacin were shown to be more efficient than enoxacin and lomefloxacin to UVA-sensitize the formation of 8-oxodGuo and Fpg-sensitive sites in the DNA of THP-1 tumoral monocytes (223). It is likely that $^1O_2$ is mainly involved in the fluoroquinolone-mediated formation of 8-oxodGuo (281), even if contribution of type I mechanism could not be totally excluded particularly for lomefloxacin, which is also able to induce reactive carbene upon UVA excitation (282,283). Rufloxacin, another antiviral fluoroquinolone, is an efficient generator of 8-oxodGuo measured by HPLC-ECD in the DNA of human nonimmortalized fibroblasts (284) and yeast strains (285) upon UVA-irradiation. Furthermore, evidence was provided that the photomutagenicity of rufloxacin in yeast cells is correlated with the formation of 8-oxodGuo (286). Preferential implication of type II photosensitization mechanism was proposed for rufloxacin from photophysical and mechanistic studies (287–289).

Therapeutic immunosuppressant, anti-inflammatory and anticancer thiopurines including azathioprine and 6-thioguanine (6-TG) prodrugs (290) are efficiently metabolized in treated patients leading to significant incorporation and accumulation in DNA of cytotoxic 6-TG nucleobases (291). The toxicity of 6-TG is greatly enhanced by solar exposure (291) being associated with mutagenic effects (292) and severe skin diseases including carcinomas (293,294) in relation with the high UVA sensitivity of 6-TG (295,296). Early evidence has shown that UVA excited 6-TG gave rise to reactive oxygen species including $O_2^{\bullet-}$ and $^1O_2$ (297). The treatment of GM5399 human diploid fibroblasts (HDFs) with azathioprine in conjunction with UVA radiation led to pronounced genotoxic effects that were assessed using the alkaline comet assay associated with an 8-oxoguanine glycosylase (OGG1) incubation step to reveal oxidatively generated guanine damage. Thus, it was shown that internal 6-TG photosensitization, being more efficient in quiescent cells than proliferating HDFs, generated in a dose-dependent manner OGG1-sensitive sites, mostly 8-oxoG together with similar amounts of DNA strand breaks and/or alkaline labile lesions (298). Indirect confirmation for the photosensitizing ability of 6-TG to trigger oxidatively generated damage to cells was inferred from the increased level of urinary 8-oxodGuo in renal transplant patients treated with immunosuppressant azathioprine (299). Direct evidence for the formation of 8-oxodGuo was provided by HPLC-ECD measurements performed on the DNA of mouse fibroblasts exposed to combined azathioprine/UVA treatment (300). Interestingly, a higher accumulation of 8-oxodGuo was observed in cell defective in MUTYH glycosylase which function is to prevent incorporation of 8-oxoG nucleoside triphosphates into DNA. The photosensitized formation of 8-oxodGuo in UVA-irradiated cells pretreated with azathioprine is rationalized in terms of efficient generation of $^1O_2$ by excited 6-TG that appears as a predominant type II photosensitizer (301). It was also shown that guanine-6-sulfonate is the main $^1O_2$ oxidation product of 6-TG both from both experimental and theoretical model studies (301,302).

Photosensitized formation of cyclobutane pyrimidine dimers

UVA-excited fluoroquinolones are able to induce damage to cellular DNA through several photosensitization mechanisms (289). In addition to photodynamic effects leading to 8-oxodGuo, several fluoroquinolones that exhibit a triplet excited energy higher or close to that estimated for thymine (E_T ~ 270 kJ mol^{-1}) (224,225) are able to trigger dimerization of vicinal pyrimidine bases according to the triplet-triplet energy transfer (TTET) mechanism. This was observed initially for lomefloxacin from the measurement of T4 endonuclease V-sensitive sites in the DNA of UVA-irradiated HaCaT human keratinocytes (303). In a subsequent study, it was found that the formation of CPDs revealed by immunodetection was three-fold more elevated in human keratinocytes than in fibroblasts (304). Evidence was also provided for the lomefloxacin mediated-photosensitization formation of CPDs in the skin of mice together with an increased cancer incidence in XPA-gene-deficient animals that lack the ability to repair bulky damage (275). In a comparative study, it was shown that enoxacin and to a lesser extent norfloxacin that have elevated E_T values (273 and 269 kJ mol^{-1}, respectively) are much more efficient than lomefloxacin and particularly ofloxacin to generate thymine cyclobutane pyrimidine dimers (T<>T) in the DNA of THP-1 human monocytes (223). A different trend is observed for isolated DNA with the lowest induction of thymine CPD for enoxacin (223) that may be partly related to a decreased photoreactivity of the fluoroquinolone when bound to calf thymus DNA (305).

Carprofen, a nonsteroidal anti-inflammatory drug, has been shown in association with UVA to sensitize the formation of CPDs in human HaCaT keratinocytes as measured by either immunofluorescence or a modified alkaline comet assay (306). Pyrimidine dimerization is likely to involve a TTET mechanism as proposed for a methyl ester of a carprofen photoproduct that shows a triplet energy value ($E_T = 269$ kJ mol^{-1}) close to thymine (307).

Chemisensitization formation of dark cyclobutane pyrimidine dimers

Exposure of melanin-containing human and murine melanocytes to either UVB or UVA radiation unexpectedly triggered a delayed formation of CPDs over at least a 4 h period after the direct generation of the dimeric lesions (308). The additional UVA-induced CPDs that were measured by ELISA, the modified alkaline comet assay and HPLC-MS/MS, represent between 50 and 75% of those initially photochemically produced. Evidence was also provided for the delayed formation of CPDs in the skin of pigmented *K14-Kitl* transgenic mice. In contrast, no generation of "dark CPDs" was observed upon UVA-irradiation of either fibroblasts or albino melanocytes that lack melanin. Implication of an oxidative mechanism in the formation of "dark CPDs" was suggested from the protective effects of *N*-acetylcysteine and tocopherol against the generation of the bipyrimidine photoproducts (308,309). It is well documented as a cellular response to UV stress that $O_2^{\bullet-}$ and nitric oxide are enzymatically produced over several hours, thus generating upon recombination reactive peroxynitrite ($ONOO^-$). It was postulated that $ONOO^-$ would react with melanin monomers giving rise to unstable dioxetane intermediates in the proximity of DNA (308,309). Subsequent thermal decomposition of dioxetanes, known from previous studies to generate triplet-excited carbonyls (310,311), is likely to induce CPDs in the dark by a TTET mechanism (308,312). The proposed stoichiometric chemiexcitation mechanism that leads to a different distribution pattern of CPDs with respect to UVA irradiation with a significant increase of C<>T and T<>C at the expense of T<>T is characterized by an ultra-weak chemiluminescence emission. However, the dioxetane precursor of the reactive carbonyls remains to be identified in at least model systems. Delayed formation of CPDs that was reported to be triggered in human keratinocytes upon exposure to UVA1 (340-400 nm) radiation was partly prevented by either pre- or post-treatment with vitamin E (313). Evidence was provided on the basis of HPLC-MS/MS measurements that "dark CPDs" are generated over a 2 h postexposure and persist for 24 h in skin of type I-III human volunteers upon exposure to a 385 nm source (314). The delayed formation of CPDs was of smaller intensity and only observed for two subjects upon visible light irradiation using a 405nm source. The dual protecting and sensitizing role of melanin on the simulated solar radiation sensitized formation of "light" and "dark" CPDs in the epidermis of Fizpatrick skin type (FST) I/II and type VI volunteers has been demonstrated (315). "Dark" CPDs were formed with a peak appearing at 1-2 h post-exposure as the likely result of melanin photosensitization mediated by oxidative reactions. In contrast, no directly produced "light" CPDs were detected in the basal layer of FST IV subjects that may be related to the UV filtering effect of melanin.

UVA-sensitized formation of psoralen cycloadducts with pyrimidine bases

Despite extensive research activities that have led to the characterization of the main psoralen monoadducts to pyrimidine bases and interstrand DNA cross-links (184,316,317), only a few attempts were made to measure the formation of these photocycloadducts in cells. This may be explained by the lack in the 80's of appropriate sensitive analytical methods for monitoring psoralen-DNA adducts at the exception of fluorescent 4',5'-furan-side monoadducts to thymidine. Thus, a sensitive HPLC-fluorescence method was designed for measuring the two *cis-syn* diastereomers of the 4',5'-furan-side adducts to thymidine (Thd<>3-CPs) in isolated DNA (195) taking advantage of suitable photophysical parameters including a fluorescence spectrum exhibiting a maximum around 425 nm with an absorption spectrum centered around 357nm (194,201). The detection threshold of the two diastereomers that show fluorescence quantum yields (Φ_f) of 0.26 and 0.37, respectively (194), was in the subpicomole range (195) thus allowing measurement of the photoadducts in cells. Using this method, the two diastereomers of Thd<>3CPs were detected in UVA-irradiated *Saccharomyces cerevisiae* yeast cells and Chinese hamster ovary V79 cells (318). The repair kinetics of the Thd<>3CPs in haploid wild-type strains N123 of *S. cerevisiae* showed similar bi-phasic curves with about 50% removal of the photoadducts after a 90 min postirradiation incubation (319). Similar repair kinetics were observed for the furan-side adducts of 7-methylpyrido[3,4-c]psoralen (MePyPs) to thymidine in the DNA of yeast cells (320). The fast removal of both 3-CPs and MPP furan-side monoadducts is compatible with the implication of the base excision repair pathway that has been shown to be involved in the NEIL 1 glycosylase-mediated removal of 8-MOP monoadducts from DNA in human cells (321). The formation of the two *cis-syn* furan-side monoadducts of bifunctional 5-methoxypsoralen to thymidine has been also monitored in *S. cerevisiae* cells using the convenient HPLC-fluorescence detection method (189). A more versatile method involving the association of the efficient HPLC separation tool with the sensitive and accurate electrospray ionization-tandem mass spectrometry that subsequently became available has been successfully developed (322,323). This allowed the detection and quantification of furan- and pyrone-side monoadducts of 8-methoxypsoralen and amotosalen S59 to thymidine in the DNA of UVA-irradiated human cells. In addition, thymidine-psoralen-thymidine bi-adducts were also measured as enzymatically released tetranucleotides.

Photosensitized oxidation of lipids and consequences to biological membranes

Hydroperoxide derivatives of unsaturated lipids (Scheme 6) alter several biophysical properties of the membranes. The tendency of the hydroperoxide moieties to migrate to the more polar environments allows for an increase in the area occupied per lipid (around 15% of area increase for a single hydroperoxide) with the consecutive decrease in the membrane thickness, increase in the membrane fluidity and decrease in the stretching module (86). The formation of hydroperoxide also alter the balance of the interfacial forces, usually increasing the level of hydrophobic mismatch and facilitating lipid demixing and domain formation (324).

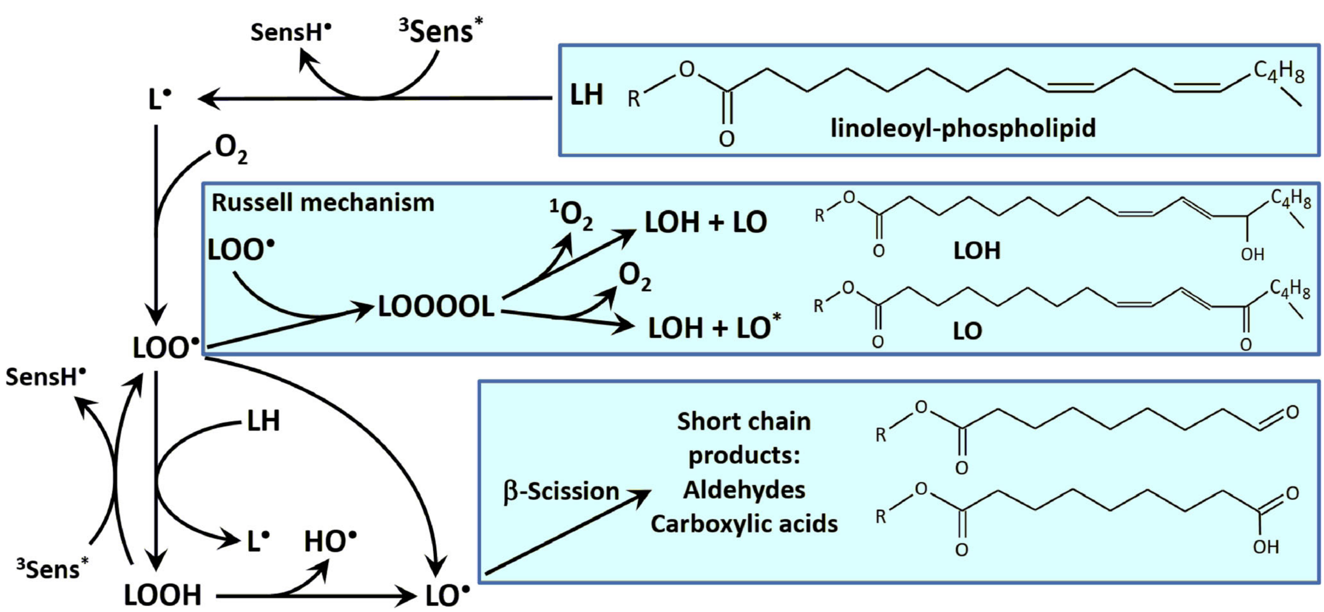
Membrane leakage depends on type I and type II mechanisms working synergistically, in order to allow several sequential steps of lipid oxidation that are necessary for membrane permeabilization. Even though the progress in the chemical analysis of oxidized lipids is not new (100), only recent studies described the molecular-level mechanisms of photoinduced membrane permeabilization, showing that lipid-truncated aldehydes are the key molecules responsible to disorganize membranes, allowing pore formation (101,325,326). The generation of lipid-truncated aldehydes occurs through β-scission reactions from lipid-derived alkoxyl radicals, which are formed by contact-dependent type I reactions from either the lipid double bond or the lipid hydroperoxide (325) (Scheme 15). Type II reaction exclusively yields lipid hydroperoxides that accumulate in the membranes without affecting permeability that facilitate several oxidation steps (100,101,325,327). However, membrane leakage correlates with an electron transfer reaction that usually causes photobleaching of the photosensitizer (70). Damage in cytoplasmic or organelle membranes is a key factor that modulates the mechanism and the overall efficiency of regulated cell death (328).

Cholesterol is highly prevalent in the membranes of mammals, exerting fundamental roles to keep their biophysical properties, working as a lipid lubricant and favoring or disfavoring lipid demixing and domain formation, which are key steps toward biological signaling (329,330). Interestingly, cholesterol is also a target of photoinduced lipid oxidation. Long ago, Girotti and coauthors realized that cholesterol hydroperoxides provide important biomarkers of the type of photoinduced lipid oxidation. At the start of the lipid oxidation, both type I and type II processes generate different types of hydroperoxide derivatives of cholesterol (ChOOHs). Free-radical type I reactions favor the attack on the carbon 7 of cholesterol, forming mainly 3b-hydroxy-cholest-5-ene-7a-hydroperoxide (7α-OOH) and 3b-hydroxycholest-5-ene-7b-hydroperoxide (7β-OOH) as primary intermediates, whereas the Type II allows the formation of three ChOOHs, which are 3b hydroxy-5a-cholest-6-ene-5-hydroperoxide (5α-OOH), 3b-hydroxycholest-4-ene-6a-hydroperoxide (6α-OOH) and 3b-hydroxycholest-4-ene-6b-hydroperoxide (6β-OOH) (331,332). These ChOOHs can be separated and quantified, allowing the identification of the initiation steps of the photo-oxidation being either by free-radicals or by $^1O_2$ even in complex systems (100). Although the first steps of the oxidation of PUFA lipids are also different comparing free-radical or $^1O_2$ initiated, the variety of compounds, its instability and the difficulties in separation/detection do not allow such an easy method to identify the oxidation mechanism.

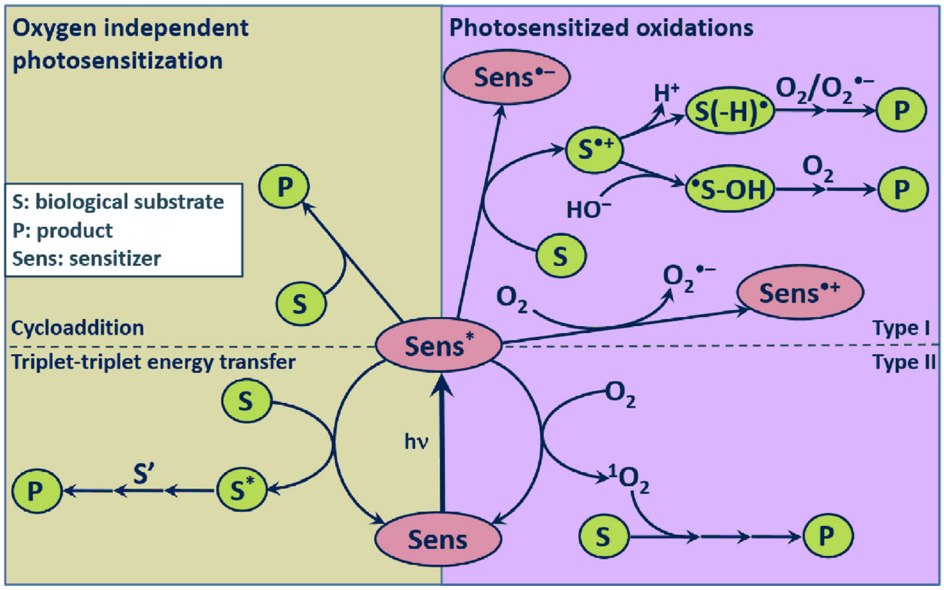
CONCLUSION

Photosensitization of biomolecules is a multidisciplinary topic with impact in environmental chemistry, biology, pharmaceutical sciences and medicine. A large number of scientists with very different backgrounds are working in this amazing field. However, the heterogeneity of the disciplines involved presents challenges in reaching a unified language. This review is an attempt to homogenize definitions, emerge to a consensus on classifications of mechanisms and provide key examples of photosensitization reactions of biomolecules and associated biological effects from UV and visible radiation. The review was written to improve understanding, where mechanistic facets were probed. We focused on type I and type II photosensitized oxidations and also offered insight into other photosensitization reactions, such as those in which oxygen is not involved. We provide information on endogenous and exogenous photosensitizers, as well as on the most important biological targets, including nucleic acids, proteins and unsaturated lipids. Definitions of photosensitized reactions are identified in biomolecules with key insight into cells and tissues.

Throughout this paper, we show the complexity of the mechanisms of photosensitized reactions, in which all are initiated by



Scheme 15. Simplified mechanism of type I photoinduced lipid oxidation leading to products different from hydroperoxides (LOOH). As an example, some representative products of linoleic acid are shown. LH, phospholipids; L•, alkyl lipid radical; LOO•, peroxyl lipid radical; LO•, alkoxyl lipid radical; LOH, hydroxy derivatives; LO, carbonyl derivative.



Scheme 16. Simplified map of the main pathways of photosensitized reactions involving biological targets.

one physical event that is the absorption of a photon by the photosensitizer. Moreover, only a few types of bimolecular reactions are possible for the excited photosensitizer as depicted in Scheme 16. The photosensitizer can react with either the biological target or O_2. This first bimolecular reaction can be a cycloaddition, an energy transfer process or an electron/hydrogen transfer process. The pathway preferred depends on the nature of the photosensitizer, the reactivity of the substrate, the experimental conditions and of the type of interactions between the two molecules. In most cases, it is difficult to predict the predominant pathway and, in general, thermodynamic and kinetic aspects have to be considered. The true complexity of the mechanism lies in the fate of the species generated in the initial bimolecular steps, that is, in the secondary reactions. We have described some of the most relevant secondary reactions for different common substrates. Indeed, many reactions can take place after the initial bimolecular processes (Scheme 16) until reaching stable products. Even under seemingly straight forward controlled experimental conditions, many competitive pathways can occur given rise to a range of products whose distribution can easily change with minor alterations in the experimental conditions.

We foresee a need for future clarity in better details of definitions as follows: (i) cellular photosensitization and associated dark pathways following exposure of cells to light. Secondary "dark" photosensitized reactions can lead to further oxidation reactions, although only marginal light emission comes from this route compared with external light sources. (ii) reactions in anaerobic environments are more prone to occur through photosensensitized reactions that require molecular contact. Further analysis can be sought that depend on excited-state sensitizer/substrate interactions. Tyrosine dimerization in the absence of oxygen is one of several examples. In some cases, the products are oxidized, but not by participation of oxygen itself. Consensus will be required with those in the community on this avenue. (iii) There remain challenges in the extrapolation of model systems to cellular systems that we expect will advance significantly in the coming few/several years. In cells, the occurrence of secondary reactions is far less expected than in model systems. There is still a strong need for sensitive and specific analytical methods for searching in cells the photosensitized formation of key modified biomolecules such as DNA-protein crosslinks.

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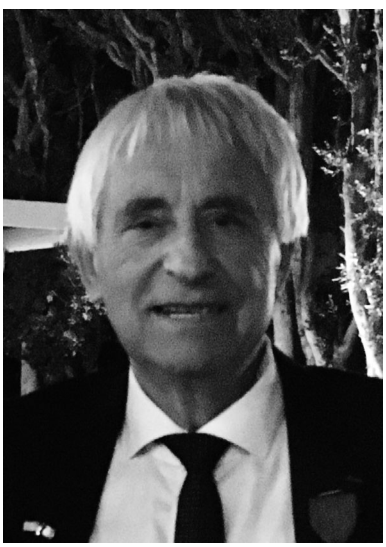

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