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Benzophenone Photosensitized DNA Damage

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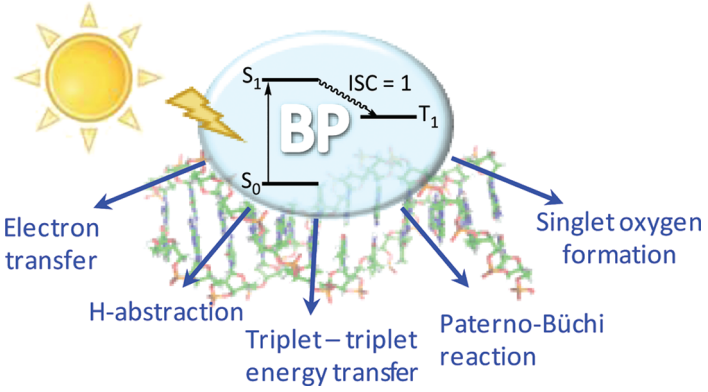

Although the carcinogenic potential of ultraviolet radiation is well-known, UV light may interact with DNA by direct absorption or through photosensitization by endogenous or exogenous chromophores. These chromophores can extend the "active" fraction of the solar spectrum to the UVA region and beyond, which means that photosensitizers increase the probability of developing skin cancer upon exposure to sunlight. Therefore researchers would like to understand the mechanisms involved in photosensitized DNA damage both to anticipate possible photobiological risks and to design tailor-made photoprotection strategies. In this context, photosensitized DNA damage can occur through a variety of processes including electron transfer, hydrogen abstraction, triplet–triplet energy transfer, or generation of reactive oxygen species.

In this Account, we have chosen benzophenone (BP) as a classical and paradigmatic chromophore to illustrate the different lesions that photosensitization may prompt in nucleosides, in oligonucleotides, or in DNA. Thus, we discuss in detail the accumulated mechanistic evidence of the BP-photosensitized reactions of DNA or its building blocks obtained by our group and others. We also include ketoprofen (KP), a BP-derivative that possesses a chiral center, to highlight the stereodifferentiation in the key photochemical events, revealed through the dynamics of the reactive triplet excited state (^{3}KP*). Our results show that irradiation of the BP chromophore in the presence of DNA or its components leads to nucleobase oxidations, cyclobutane pyrimidine dimer formation, single strand breaks, DNA–protein cross-links, or abasic sites. We attribute the manifold photoreactivity of BP to its well established photophysical properties: (i) it absorbs UV light, up to 360 nm; (ii) its intersystem crossing quantum yield (ϕ_{ISC}) is almost 1; (iii) the energy of its nπ^* lowest triplet excited state (E_T) is ca. 290 kJ mol^{-1}; (iv) it produces singlet oxygen ($^1O_2$) with a quantum yield (ϕ_Δ) of ca. 0.3.

For electron transfer and singlet oxygen reactions, we focused on guanine, the nucleobase with the lowest oxidation potential. Among the possible oxidative processes, electron transfer predominates. Conversely, triplet–triplet energy transfer occurs mainly from ^{3}BP* to thymine, the base with the lowest lying triplet state in DNA. This process results in the formation of cyclobutane pyrimidine dimers, but it also competes with the Paternò–Büchi reaction in nucleobases or nucleosides, giving rise to oxetanes as a result of crossed cycloadditions. Interestingly, we have found significant stereodifferentiation in the quenching of the KP triplet excited state by both 2′-deoxyguanosine and thymidine. Based on these results, this chromophore shows potential as a (chiral) probe for the investigation of electron and triplet energy transport in DNA.

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1. Introduction

Photochemical DNA damage is currently a matter of public health concern.[1,2] This adverse effect can be induced by direct absorption of UV light or through indirect light absorption by endogenous or exogenous chromophores near the biomacromolecule. By extending the "active" fraction of solar radiation to the UVA and beyond, photosensitizers increase the risk of developing skin cancer upon exposure to sunlight. For this reason, it is of paramount importance to understand the mechanisms involved in photosensitized formation of DNA damage, in order to develop efficient photoprotection strategies.

Benzophenone (BP) is a classical and paradigmatic sensitizer in photochemical studies. Irradiation of this chromophore in the presence of DNA leads to formation of nucleobase modifications, cyclobutane pyrimidine dimers (CPDs), DNA−protein cross-links, single strand breaks (ssb), or abasic sites. The photophysical properties of BP have been intensively studied and are well established (Figure 1): (i) it absorbs UV light, up to 360 nm, (ii) its intersystem crossing quantum yield (ϕ_{ISC}) is near 1, (iii) the energy of its nπ^* lowest triplet excited state (E_T) is ca. 290 kJ mol^{-1}, and (iv) it produces singlet oxygen ($^1O_2$) with a quantum yield (ϕ_Δ) of ca. 0.3.[3,4]

In this Account, we use BP to illustrate the advances in the investigation of the reaction mechanisms involved in photosensitized DNA damage, paying special attention to stereodifferentiation. Detailed information is provided on the main photoinduced reactions of DNA mediated by BP and related derivatives like ketoprofen (KP), a 2-arylpropionic acid with a BP chromophore that possesses a chiral center.[5,6] These reactions include triplet−triplet energy transfer (TTET) to nucleobases, together with both type I (hydrogen atom or electron transfer) and type II (singlet oxygen) processes.[7]

2. Benzophenone Photosensitized Reaction of Pyrimidine (Pyr) Bases: Triplet−Triplet Energy Transfer (TTET)

Photosensitized TTET may occur from BP to the nucleobases, especially to thymine (Thy), which is the DNA base with the lowest E_T (310 kJ mol^{-1}).[8] Subsequent reaction of ^{3}Thy* with another Thy or a cytosine (Cyt) in their ground states, gives rise to CPDs through a [2 + 2] photocycloaddition (Figure 2). As a result, a number of regio- and diastereoisomers can be obtained in solution with free 2′-deoxyribonucleosides, although there is certain prevalence of the *trans−anti* forms.[9]

In complex systems like oligonucleotides or DNA itself, the scenario is different. Thus, photosensitization of

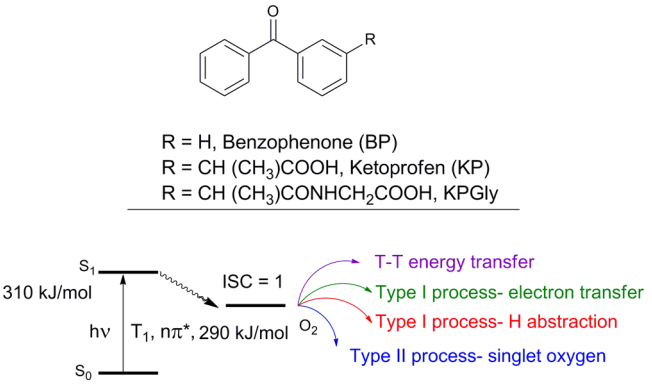

FIGURE 1. Photophysical properties and photoreactions of the benzophenone chromophore.

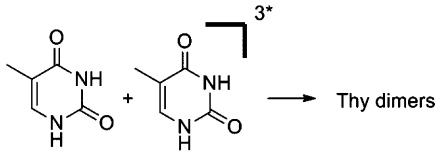

FIGURE 2. Thymine base dimerization.

oligonucleotides and ss-DNA gives mainly rise to *cis−syn* and *trans−anti* cyclobutane thymine dimers (Thy<>Thy), while in ds-DNA *cis−syn* CPDs clearly predominate[10] due to orientation restrictions imposed by the double strand.

Analysis of CPD formation photoinduced by BP in calf thymus DNA reveals a relative distribution of Thy<>Thy, 5′-Cyt<>Thy-3′ and 5′-Thy<>Cyt-3′ of 1, 0.23, and 0.25, respectively.[11] Cyclobutane cytosine dimers (Cyt<>Cyt) are not detected likely because ^{3}BP* is not energetic enough to populate ^{3}Cyt* (334 kJ mol^{-1}).[9] Absolute photodimerization quantum yields (ϕ_D) are difficult to obtain experimentally given that it has to be ensured that light is absorbed *exclusively* by the photosensitizer. For this reason, there are only a few ϕ_D values in the literature, one of them corresponding to ketoprofen; specifically, ϕ_D (KP) in supercoiled DNA has been determined to be 0.0002.[12]

According to their relative triplet energies, TTET between ^{3}BP* and Thy is a slightly disfavored process, yet it is still observed in solution due to thermal population of upper vibrational states of ^{3}BP*.[8,9,13] Notably, this process is more feasible in DNA, where π-stacking and base pairing result in a shift of the E_T of Thy down to 267 kJ mol^{-1} (Figure 3).[9,12,14,15]

We have determined the triplet energy of Thy in DNA by photosensitization experiments, in which supercoiled DNA is irradiated in the presence of a family of fluoroquinolones. The known E_T values of these drugs are within a narrow

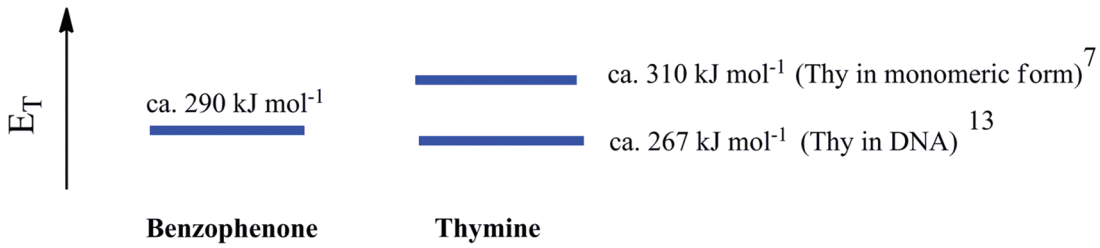

FIGURE 3. Benzophenone and thymine triplet energy levels.

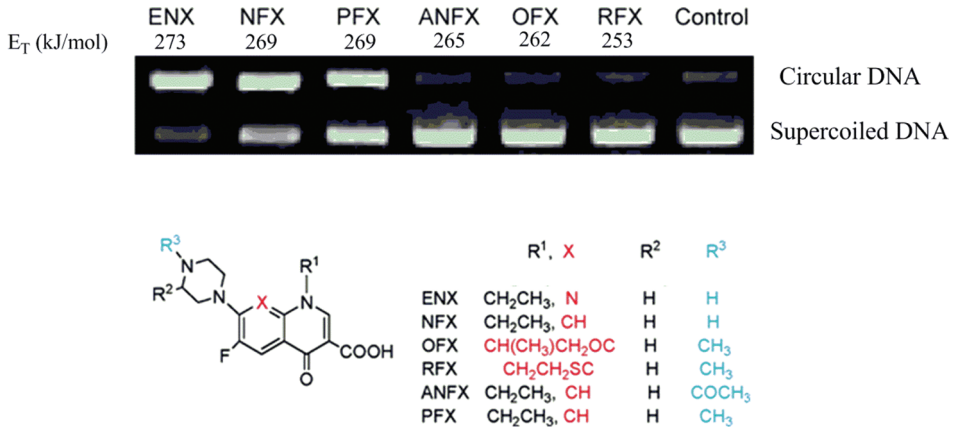

FIGURE 4. Photomixtures of fluoroquinolones of known E_T and plasmid pBR322 DNA after treatment with T4 endo V enzyme and gel electrophoresis.

range (from 273 to 253 kJ mol^{-1}), close to the expected E_T of Thy in the biomacromolecule. Following UVA irradiation, the samples are digested with T4 endonuclease V, which cleaves the double helix at those points where Thy<>Thy are formed, converting supercoiled DNA into its circular form. Subsequently, Thy<>Thy are revealed by electrophoresis, based on the different mobility of supercoiled and circular DNA (Figure 4). In this way, we have clearly shown that those drugs with $E_T > 269$ kJ mol^{-1} photoinduce Thy<>Thy, while those with $E_T < 265$ kJ mol^{-1} do not. Hence, any compound with $E_T > 267$ kJ mol^{-1} should be considered as a potential photosensitizer via Thy dimerization. This value is higher than the E_T of other well-known DNA photosensitizers, such as riboflavin (ca. 200 kJ mol^{-1}).[16]

Furthermore, studies performed on oligonucleotides have demonstrated that CPD formation is sequence-dependent.[12,17−20] In particular, the amount of these lesions increases when an additional Pyr base is located in the 5′ side of two consecutive Thy as shown by irradiation of 5′-TGA GCG TTA GTT TAA GTC GGC TAT C-3′ in the presence of BP, which leads to the highest CPD formation yields at the TTT sites.[12]

Competing with TTET, the contribution of the type I mechanism to photoinduce DNA damage has been

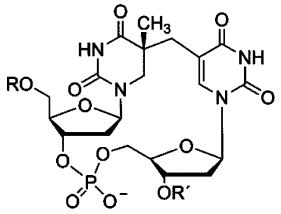

FIGURE 5. Structure of the spore photoproduct.

evaluated by irradiating BP in the presence of the dinucleotide thymidylyl-(3′→5′)-thymidine (TpT) under aerobic conditions.[10] By quantification of Thy<>Thy dimers, we have shown that the energy transfer mechanism clearly predominates over Thy oxidation (17:1 ratio).

Another structurally interesting type of Pyr dimer, found in the dry environment of bacterial spores, is the 5-thyminyl-5,6-dihydrothymine adduct, commonly known as spore photoproduct (SP, Figure 5).[9,11,21,22] The formation of this bipyrimidine lesion can be photosensitized by BP in dry films.[22] The photosensitized formation of SP in DNA gives rise uniquely to the 5*R* diastereomeric form and is conditioned by the presence of α/β acid soluble protein, which converts β-DNA into α-DNA. In the spores, dipicolinic acid seems to play the role of a natural photosensitizer.

After generation of ^{3}Thy* by TTET, we have proposed two alternative mechanisms of SP formation: (i) C−C coupling of a radical pair generated by H-abstraction from a ground state Thy and, less likely, (ii) a concerted mechanism.[22,23]

3. Benzophenone Photoreaction with Pyrimidine Bases: The Paternò−Büchi Reaction

Carbonyl compounds may react with olefins through a [2 + 2] photocycloaddition giving rise to oxetanes through a Paternò−Büchi reaction (Figure 6). This competes with TTET and is favored for nπ* triplets when the E_T of the alkene is comparable to or higher than that of the carbonyl compound. Because this is the case for the BP/Thy system, oxetane formation is possible.[3,13,24]

Actually, upon irradiation of BP in the presence of thymidine (Thd), we have isolated two stereoisomeric oxetanes (Figure 7).[24]

To gain a deeper insight into the reaction mechanism, we have performed time-resolved laser flash photolysis (LFP) experiments to study the interaction between the triplet excited states of BP or KP and Thd. Because both ^{3}BP* and ^{3}KP* are nπ* in nature, a fast triplet−triplet quenching by Thd is observed, (ca. 5.0 × 10^8 M^{-1} s^{-1}). This supports a Paternò−Büchi photoreaction,[24] in view of the endergonic nature of TTET. Accordingly, oxetanes prevail over CPDs after steady-state irradiation of Thy in the presence of BP.[8,10,24] Indeed, BP-photosensitized Thy dimerization is concentration dependent, and CPDs are only detected when the nucleobase is present in a large excess.

It is worth noting that this scenario may vary in DNA, where the contribution of TTET would be higher, due to the lower E_T of Thy in the biomacromolecule. Thus, the double helix would prevent the Paternò−Büchi photoreaction from taking place but at the same time would enhance the prospects for Thy dimerization.

3.1. Chiral Discrimination. Direct photophysical evidence for chiral discrimination in the triplet excited state has only been found in a few cases;[13,25−29] this includes the interaction between ^{3}KP* and Thd, which we have studied by LFP in aqueous acetonitrile, monitoring the kinetics of KP nπ* triplet state decay upon addition of increasing amounts of Thd.[13] Plotting the reciprocal lifetimes of (*S*)- and (*R*)-^{3}KP* vs Thd concentration, we obtained quenching rate constants of $k_S = 3.6 \times 10^8$ M^{-1} s^{-1} and $k_R = 5.1 \times 10^8$ M^{-1} s^{-1} for (*S*)- and (*R*)-KP, respectively (Figure 8).

We have investigated the intramolecular version of this reaction in the *cisoid* (5′-KP-Thd) or *transoid* (3′-KP-Thd) dyads (Figure 9) where KP is attached to positions 5′ or 3′ of the 2-deoxyribose moiety.[30]

Long wavelength irradiation of the *transoid* form leads to polymerization. Conversely, a mixture of photoproducts is obtained from the *cisoid* isomer, where the oxetanes arising from a Paternò−Büchi reaction (Figure 10) are clearly predominating (combined yield of ca. 52%). In addition, minor

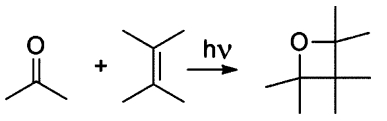

FIGURE 6. The Paternò−Büchi reaction.

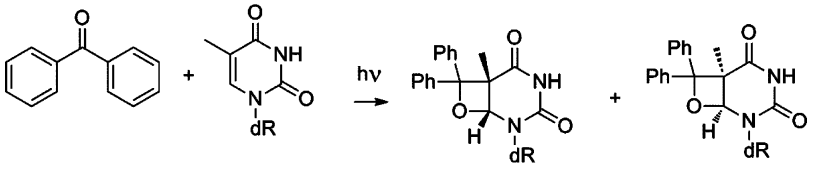

FIGURE 7. Oxetane formation upon irradiation of BP and Thd.

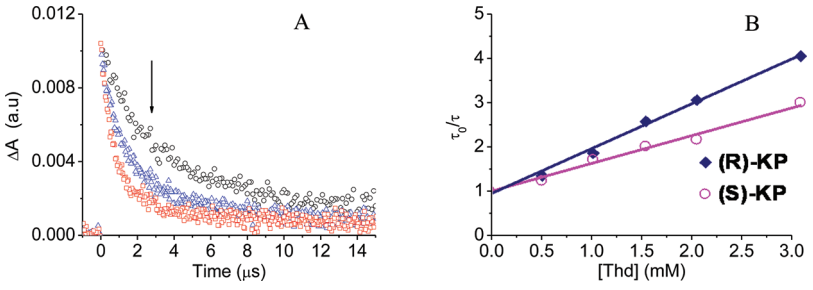

FIGURE 8. (A) Ketoprofen triplet excited state decay upon addition of increasing amounts of Thd using MeCN/H_2O (4:1, v/v) as solvent and (B) Stern−Volmer plots for quenching of (*R*)- and (*S*)-^{3}KP* by Thd.

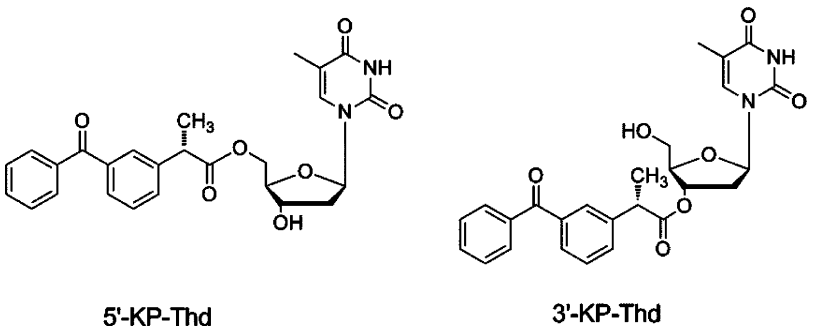

FIGURE 9. Ketoprofen–thymidine dyads.

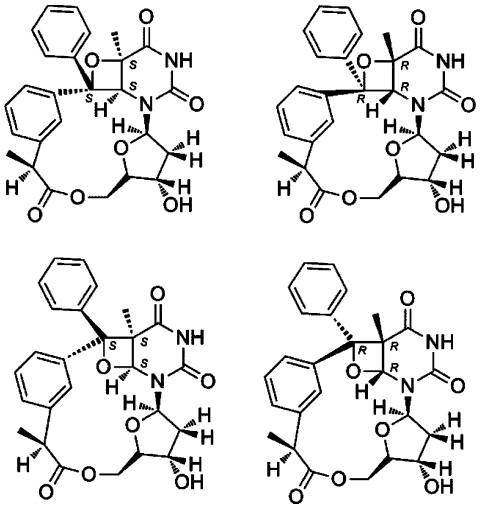

FIGURE 10. Photoproducts isolated from irradiation of the *cisoid* 5′-KP-Thd dyad.

amounts of products resulting from initial hydrogen abstraction by the excited ketone from the 5-methyl group of Thy are also detected.

Our results showed a good correlation between the photoproduct yields and the LFP measurements. Thus, the transient absorption spectra of the dyads essentially coincide with the TT bands of (*S*)-KP, displaying two maxima centered at 330 and 530 nm (Figure 11). However, the triplet lifetimes of the reference compound, τ_T((*S*)-KP) = 1.3 μs, and the dyads are strikingly different. This is particularly noteworthy in the case of the *cisoid* form whose τ_T is 20 ns, much shorter than the value obtained for the *transoid* isomer (τ_T = 300 ns, Figure 11).

4. Benzophenone-Photosensitized Type I Oxidation

In addition to its above-mentioned capability to photosensitize the formation of Thy lesions by TTET and Paternò–Büchi reaction, BP is also able to oxidize DNA. The ability of BP to photosensitize oxidatively generated DNA damage is extensively reported in the literature.[31−38] Most of the published work deals with an electron transfer mechanism triggered by BP in its triplet excited state. Indeed, the Rehm–Weller equation allows determination of free energy changes of −70 and −30 kJ mol^{-1} for the reaction with 2′-deoxyguanosine (dGuo) and Thd, respectively.[12] Nonetheless, although ^{3}BP* is in principle able to oxidize all nucleobases, a particular emphasis has been placed on dGuo, the nucleoside with the lowest oxidation potential. When BP is compared with a typical DNA type I photosensitizer, such as riboflavin, the latter exhibits a lower oxidizing ability, with free energy changes ca. 30 kJ mol^{-1} more positive than BP.[16] Thus, both compounds mediate one-electron oxidation of guanine (and to a lesser extent adenine) in double-stranded DNA; however, thymine oxidation has only been reported for BP.[39]

4.1. Reaction with Purine Bases: An Electron Transfer Mechanism. Information on the primary processes involved in the interaction between excited BP and dGuo is provided by LFP studies. Thus, the decay kinetics of ^{3}BP* (or its derivatives KP and KPGly, Figure 1) in the presence of dGuo demonstrates a high reactivity, with a bimolecular rate constant close to diffusion ($k_q > 10^9$ M^{-1} s^{-1}).[13,40,41] Moreover, we have confirmed the electron transfer nature of the process by detection of ketyl radical (KPGly(H$^•$)), obtained by protonation of the initially formed KP radical anion, together with the neutral dGuo(−H)$^•$ radical (Figure 12).[40]

Our results revealed a stereodifferentiating interaction between enantiopure (*S*)- or (*R*)-KP triplet excited state and dGuo, for which we determined quenching rate constants of k_S(dGuo) = 1.00 × 10^9 M^{-1} s^{-1} and k_R(dGuo) = 1.23 × 10^9 M^{-1} s^{-1} in aqueous acetonitrile. This agrees well with the relative amounts of (*R*)- and (*S*)-KP ketyl radical formation (Figure 13).

Steady-state irradiation studies also point to a type I mechanism. As a first clue, the hallmark of an electron transfer process is observed in double-stranded oligonucleotides

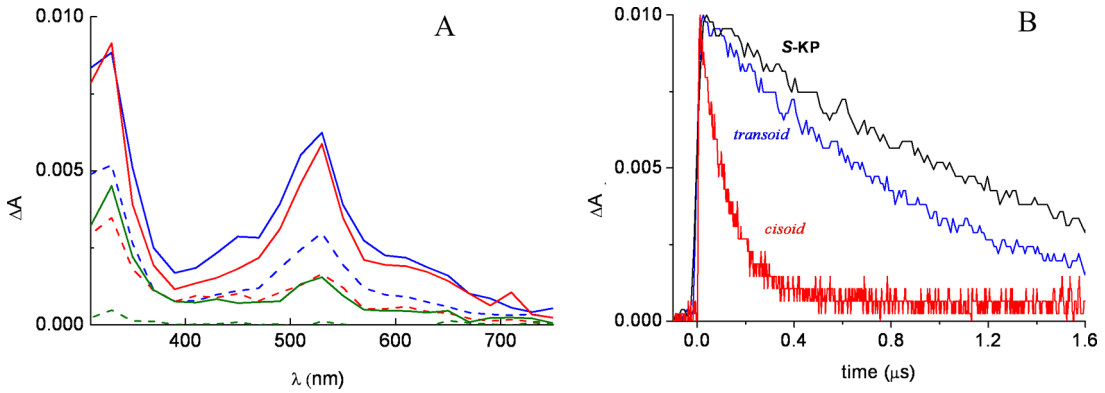

FIGURE 11. (A) Transient absorption spectra of the dyads and (*S*)-KP in acetonitrile, 35 ns (full line) and 2 μs (dashed line) after laser excitation and (B) triplet excited states of (*S*)-KP and the *cisoid* (3′-KP-Thd) and *transoid* (5′-KP-Thd) dyads.

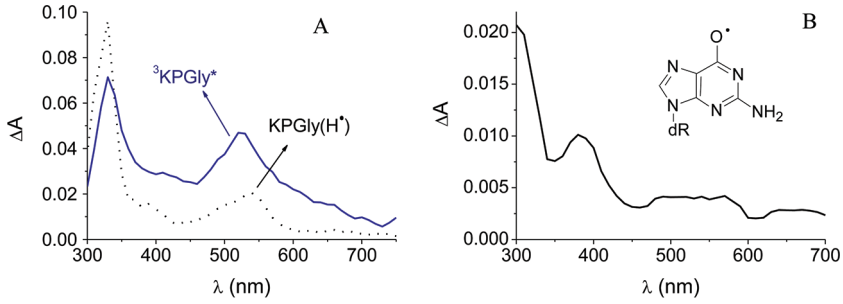

FIGURE 12. (A) Benzophenone-like triplet excited state (full line) and ketyl radical (dotted line) together with (B) dGuo(−H)$^•$ radical obtained by laser flash photolysis of KPGly/dGuo mixture in neutral aqueous medium (phosphate buffer).

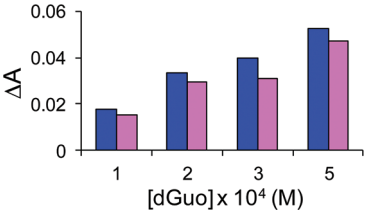

FIGURE 13. Comparison of the amount of ketyl radical formed after flash excitation of a solution of enantiopure (*S*)-KP (pink) or (*R*)-KP (blue) in the presence of dGuo, using MeCN/H_2O (4:1, v/v) as solvent.

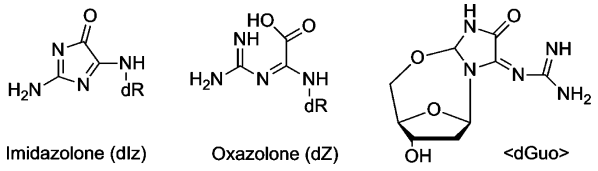

FIGURE 14. Structures of imidazolone and oxazolone, the typical product for BP-photosensitized type I oxidation of dGuo, together with the intrabase product <dGuo>.

irradiated in the presence of BP. Gel sequencing experiments show a highly specific alkali-labile site at the hot spot 5′-G of -GG- and in the middle G of -GGG- sequences.[12,36,42] Moreover, prolonged irradiation leads to degradation of all G residues, with efficiency decreasing in the order 5′-GG > 5′-GA > 5′-GC > 5′-GT, in good agreement with the calculated ionization potentials of stacked nucleobase models.[12] The capability of BP to act as a strong electron acceptor has been exploited to attach covalently this chromophore to predetermined sites of oligodeoxynucleotides, without perturbing the base stack, in order to investigate hole migration to remote sites.[42] This principle can be applied to the development of new probes for the study of electron transport in DNA.

In the case of isolated dGuo, typical photoproducts derived from electron transfer from the nucleobase to ^{3}BP* are mainly obtained. They correspond to the unstable 2-amino-5-[(2-deoxy-β-D-*erythro*-pentofuranosyl)amino]-4*H*-imidazol-4-one (dIz), which is further hydrolyzed to 2,2-diamino-4-[(2-deoxy-β-D-*erythro*-pentofuranosyl)amino]-5(2*H*)-oxazolone (dZ) (Figure 14).[41,43−46] Interestingly, we also obtained photoproduct <dGuo> based on an intrabase link as a result of a primary electron transfer, followed by nucleophilic attack by the 5′ hydroxyl group to the C8 position of the nucleobase (Figure 14).[47]

In similar studies on the dinucleotide thymidylyl-(3′→5′)-2′-deoxyguanosine (TpdG), we described the corresponding oxazolone product (TpdZ) as the main photoproduct,

together with a 2-deoxy-D-ribono-1,4-lactone derivative TpdL.[48] This sugar oxidation, also reported in the case of dGuo, is of special interest because it leads to the formation of an oxidized abasic site. The proposed mechanism is based on electron transfer oxidation of the nucleobase, followed by deprotonation at C1′ of the guanine radical cation giving rise to a neutral radical, which after oxygen trapping, release of superoxide radical anion, and hydration of the resulting 2-deoxyribose cation gives rise to 2-deoxy-D-ribono-1,4-lactone (dL) (Figure 15).[49] However, direct hydrogen abstraction cannot be totally discarded as initial step. Mechanistic confirmation has been provided by combining photoproduct characterization and time-resolved experiments with appropriate model systems.

Thus, the KP−purine dyads shown in Figure 16 have been first considered.[50] Their structural variations have allowed us to evaluate the different factors influencing the electron transfer mechanism. In this way, changes associated with the *cisoid* versus *transoid* spatial arrangement have been investigated with dyads 5′-KP-dAdo and 3′-KP-dAdo respectively, while compounds 5′-KP-dGuo, 5′-KP-dAdo, and 5′-KP-8-oxodAdo have been chosen to obtain information on the relative base reactivity. In addition, the length of the spacer has also been considered by comparing 5′-KP-dAdo with 5′-KPGly-dAdo. The experimental results fulfilled our expectations for an electron transfer from the purine to ^{3}KP*. As a first piece of evidence, only *cisoid* 5′-KP-purines lead to the formation of a 2-deoxyribonolactone (5′-KP-dL, Figure 16) as major photoproduct. Accordingly, while triplet lifetime of the *transoid* 3′-KP-dAdo is similar to that of isolated KP, used as standard, a much faster decay is observed for 5′-KP-dAdo. In general, we determined lifetimes in submicrosecond range for all the 5′-KP-purines in agreement with an efficient interaction between the excited KP and the nucleobase. As a matter of fact, the intramolecular quenching rate constants, ranging from 3.3×10^7 s^{-1} for 5′-KP-dAdo to 1.1×10^8 s^{-1} for 5′-KP-dGuo, correlate well with the one-electron oxidation potentials of nucleobases. Additional evidence is provided by the influence of the spacer length, which results in a markedly lower reaction rate constant for 5′-KPGly-dAdo (ca. 2.2×10^6 s^{-1}) than for 5′-KP-dAdo.

The behavior of diastereoisomeric (*S*,*S*)- and (*S*,*R*)-KP-THF conjugates bearing tetrahydrofuran as a base-free model of the 2-deoxyribose moiety (Figure 17) allowed us to rule out the possibility of a direct H-abstraction from the sugar at C1′.[51] Kinetic analysis of the transient absorption spectra reveals that the (*S*,*S*)-KP-THF triplet signal decays

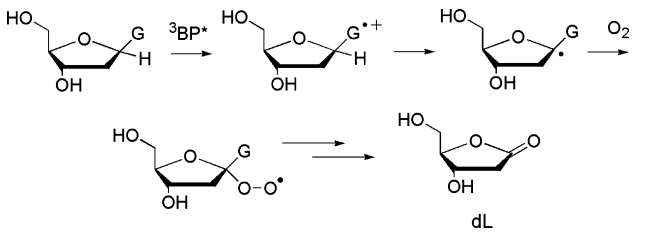

FIGURE 15. Mechanism of 2-deoxyribonolactone (dL) formation.

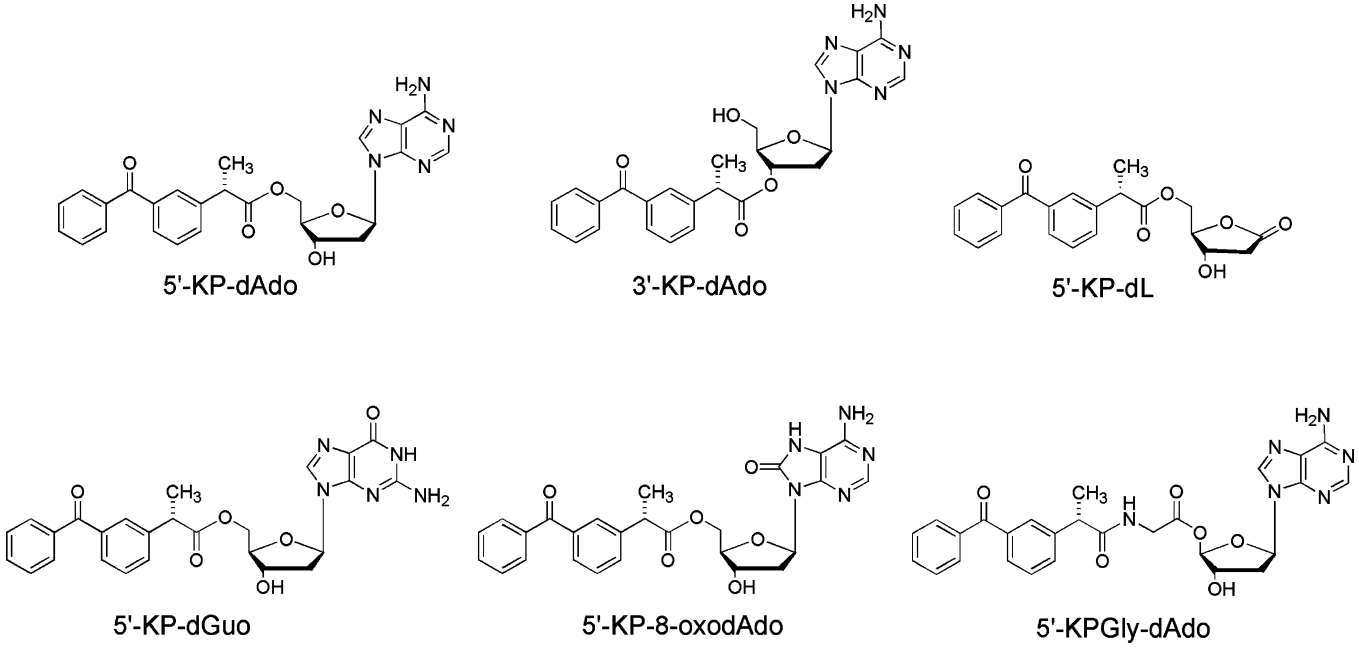

FIGURE 16. Structure of KP−purine dyads and 5′-KP-dL.

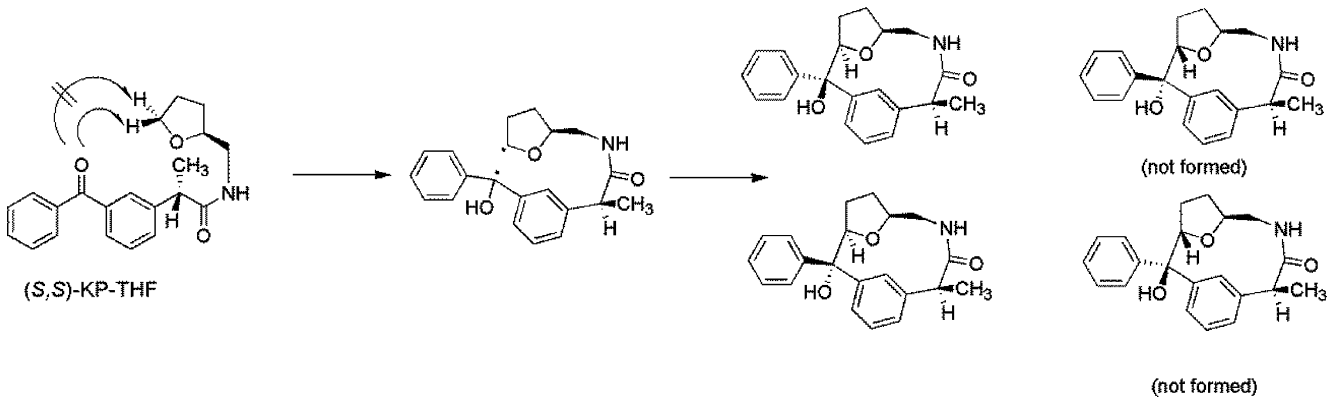

FIGURE 17. Structure and reactivity of the (*S*,*S*)-KP-THF.

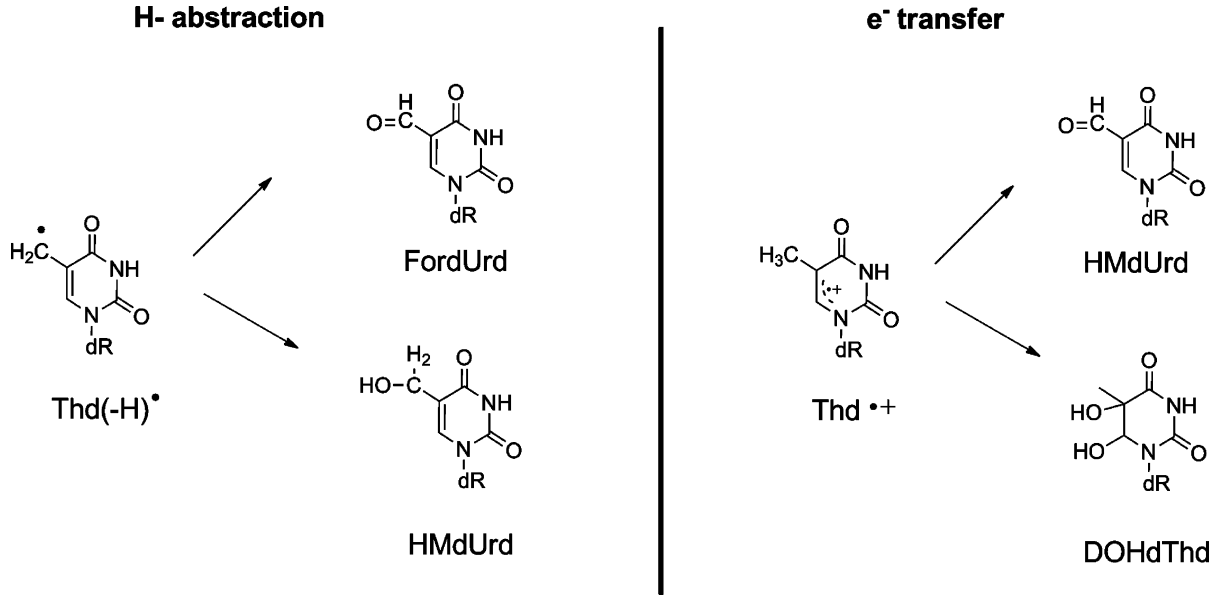

FIGURE 18. Photooxidation of Thd by BP.

significantly faster than that of the (*S*,*R*)-isomer. Moreover, the reaction rate constants of 5.9 and 3.2 $\times$ 10^5 s^{-1} are at least 2 orders of magnitude lower than for the 5′-KP−purine dyads. This demonstrates that a different primary process is involved in the photochemistry of these two types of systems. We have obtained the same conclusion from photoproduct studies, where biradicals initially formed via remote hydrogen abstraction undergo intramolecular recombination to macrocyclic ring systems with high regio- and stereoselectivity (Figure 17). In all cases, the products with *cisoid* ring junction are preferentially or even exclusively obtained, in agreement with their smaller ring strain.

Altogether our results are consistent with the predominance of an electron transfer mechanism during the BP-photosensitized oxidation of purine nucleosides to dL as detailed in Figure 15.

4.2. Reaction with Pyrimidine Bases: One-Electron Oxidation, H-Abstraction and Intrabase Cross-Link. In addition to the Paternò−Büchi photoreaction and the TTET between ^{3}BP* and Thd, oxidation of Thd may occur as a secondary reaction, given the ability of the chromophore to abstract hydrogen or to participate in electron transfer processes.[10,45] We have studied this photoreaction in aerated medium and identified the products as 5,6-dihydroxy-5,6-dihydrothymidine diastereomers (DOHdThd), 5-(hydroxymethyl)-2′-uridine (HMdUrd) and 5-formyl-2′-deoxyuridine (FordUrd) (Figure 18). Formation of a neutral radical centered on the 5-methyl of Thd after a formal H-abstraction by the excited ketone or deprotonation of thymine radical

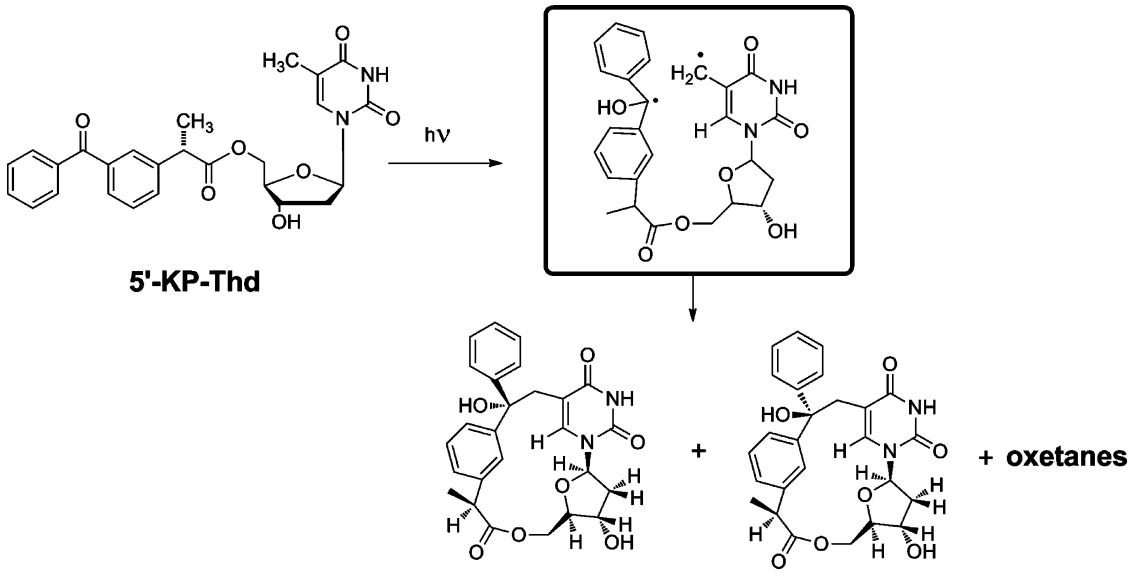

FIGURE 19. Hydrogen abstraction in the photoreaction of the *cisoid* 5′-KP-ThdKP-BP dyad.

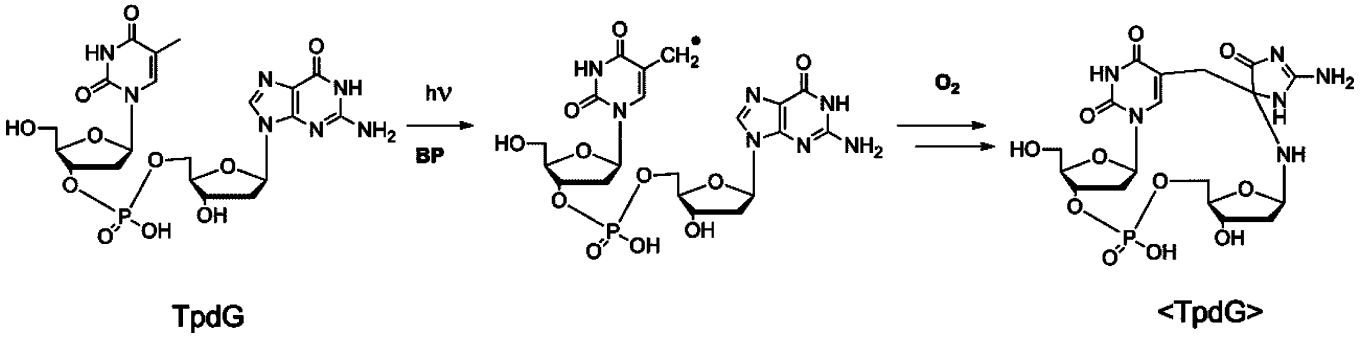

FIGURE 20. Photosensitization of TpdG by BP under aerated conditions.

cation at the methyl group leads to FordUrd and HMdUrd, while DOHdThd arises from hydration of Thd radical cation. The former pathway is in agreement with LFP results, while the presence of the four DOHdThd diastereomers in the reaction mixture supports the formation of Thd radical cation.

We have observed hydrogen abstraction at the C-5 of the base by ^{3}BP* upon irradiation of the *cisoid* KP-Thd dyad presented in the TTET (section 3.1), which leads to a couple of minor products (14% combined yield, Figure 19) arising from recombination of a primary biradical.[30]

Type I reactions induced by BP have also been assessed in TpdG dinucleotides.[45] In our hands, photosensitization of TpdG in the presence of BP leads to formation of an adduct (<TpdG>, Figure 20) resulting from formal hydrogen abstraction at the C-5 of the Thy base by ^{3}BP*. Generation of a carbon-centered radical would be the first step in a sequence of reactions ultimately producing a covalent linkage to the C-4 of the guanine.

4.3. Modeling DNA−Protein Cross-Links. In eukaryotic cells, DNA−protein cross-links are important contributors to the deleterious effects of solar radiation, because of the close contact between DNA and proteins such as histones. Thus, the role of type I oxidation in the formation of these adducts has been investigated using BP as photosensitizer and dGuo as a simple unit of the DNA biomolecule.

In this context, BP-photosensitized reaction between dGuo and the methyl ester of acetylated lysine leads to the spiroiminodihydantoin derivative 8-Lys-Sp as the main photoproduct, together with small amounts of 5,8-Lys-Sp (Figure 21A).[52] These compounds are the result of an electron transfer process leading to covalent adduct formation between the ε-amino group of lysine and the C8 position of the nucleobase, which further undergoes rearrangement to give the spirocyclic adducts. We have also used methanol as a mimic of the hydroxyl group of tyrosine, threonine, or serine side chain. In this case, two 4,5-imidazolidinedione diastereoisomers are obtained as

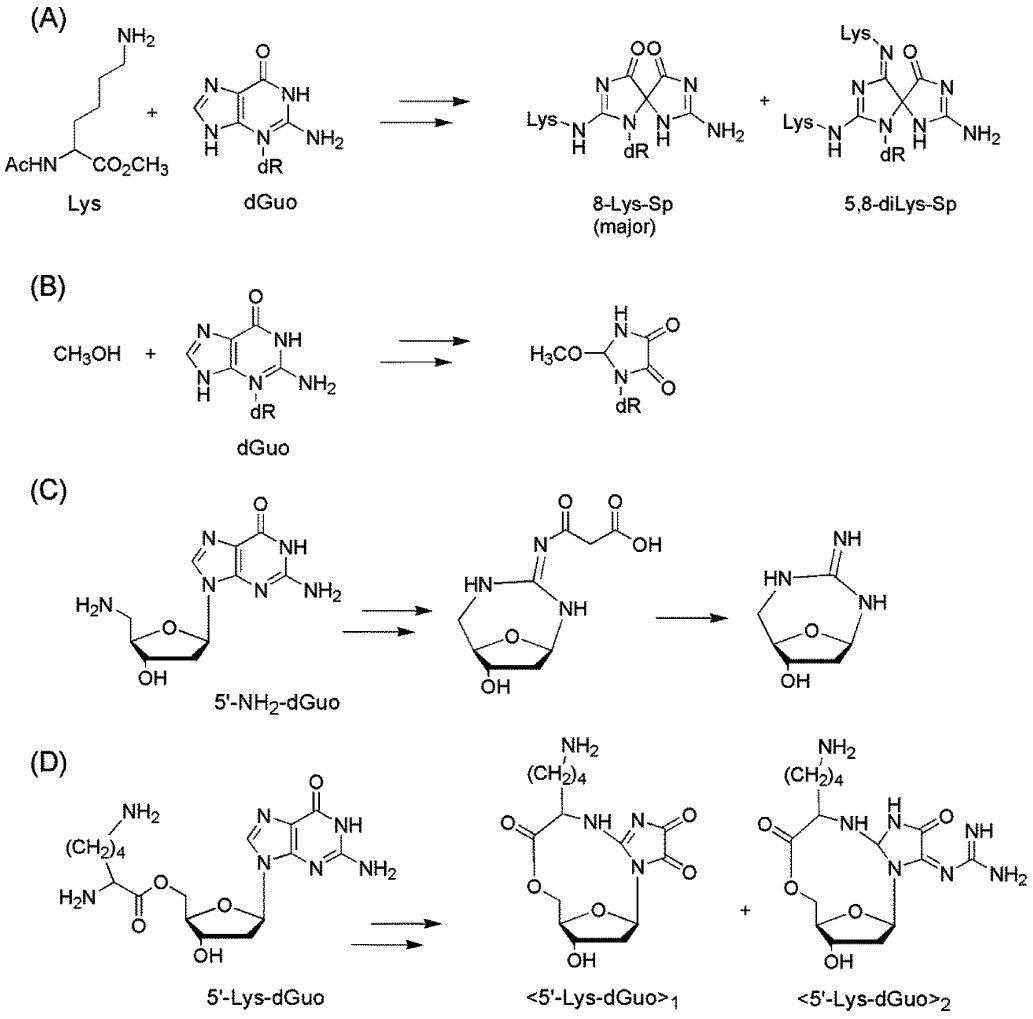

FIGURE 21. Model photoreactions for the BP-sensitized DNA–protein cross-links.

products of the nucleophilic addition of methanol to the guanine base (Figure 21B).[44]

Furthermore, we have modeled the intimate association between DNA and histones using different systems containing an amino group or a lysine residue tethered at the C5′ of dGuo. Thus, BP mediated oxidation of 2′-amino-2′,5′-dideoxyguanosine (5′-NH_2-dGuo, Figure 21C)[53] in aerated aqueous solution leads to the formation of two cyclic nucleosides, where the heterocyclic guanine ring is missing (Figure 21C). In the case of a lysine residue linked at C5′ of dGuo (5′-Lys-dGuo, Figure 21D), two intramolecular adducts are formed in low yield (ca. 2%).[54] Although both compounds derive from a reaction between the α-NH_2 of lysine and the C8 position of electron transfer oxidized guanine, <5′-Lys-dGuo>$_1$ would be formed by a nucleophilic attack to the guanine radical cation, whereas <5′-Lys-dGuo>$_2$ can be explained by addition of the α-NH_2 group to the 7,8-double bond of the neutral dGuo radical.

5. Type II Processes: Singlet Oxygen

A photosensitizer in its triplet excited state may interact with molecular oxygen, generating $^1O_2$, which is a very potent oxidizing agent. This is the case for BP and KP; they produce $^1O_2$, which in turn reacts with guanine yielding spiroiminodihydantoin diastereoisomers or 8-oxodGuo, in double stranded DNA (Figure 22). The ability of this reactive species to photoinduce DNA lesions through a type II mechanism has been examined in aqueous solutions, in the presence of single-stranded oligonucleotides. When D_2O is used instead of H_2O, the BP-photosensitized DNA damage increases, indicating that, to a certain extent, a type II mechanism is involved.[12]

Nevertheless, dGuo sensitization studies indicate that BP-mediated photooxidation is dominated by the type I mechanism.[41,45] Consistently, dGuo conversion upon UVA irradiation in the presence of BP is not affected by the presence of D_2O and is lower in aerated solution.

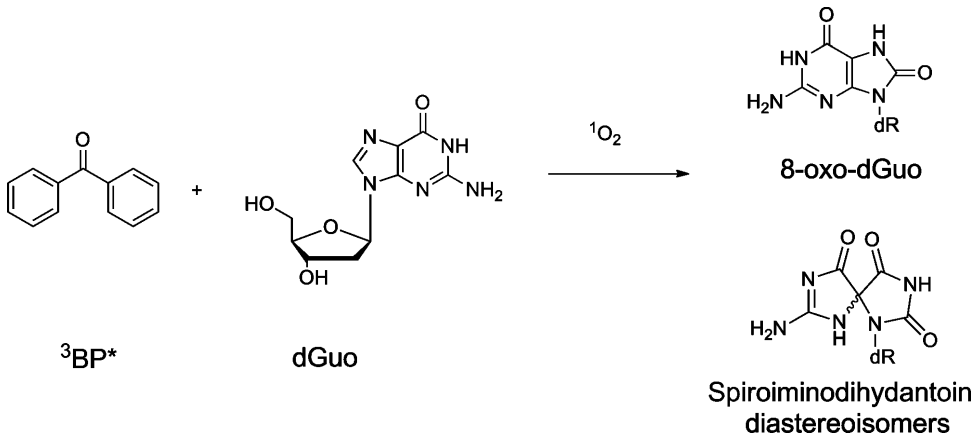

FIGURE 22. Type II photooxidation of dGuo by BP.

6. Summary and Outlook

Light is a potentially carcinogenic agent. For this reason, it is of paramount importance to understand the mechanisms involved in photoinduced DNA damage, in order to develop efficient photoprotection strategies. Ultraviolet radiation can interact with the biomacromolecule by direct light absorption or through photosensitization by endogenous or exogeneous chromophores, which extend the "active" fraction of the solar spectrum to the UVA and beyond. As a consequence, photosensitizers increase the risk of developing skin cancer upon exposure to sunlight. Photosensitized DNA damage may occur through processes comprising electron transfer, hydrogen abstraction, triplet–triplet energy transfer, or reactive oxygen species generation.

Here, we have chosen benzophenone (BP) as a classical and paradigmatic chromophore to illustrate the different lesions that photosensitizers may provoke in systems of increasing complexity: nucleosides, oligonucleotides, or DNA itself. Thus, we provide detailed mechanistic information on the main photoinduced reactions of DNA mediated by BP. Related derivatives like ketoprofen (KP), a BP-like compound that possesses a chiral center, have been included to highlight the possibility of stereodifferentiation. In this context, irradiation of the BP chromophore in the presence of DNA or its building blocks leads to nucleobase oxidations, cyclobutane pyrimidine dimers formation, single strand breaks, DNA–protein cross-links or abasic sites. The manifold photoreactivity of BP is attributed to its well established photophysical properties: (i) it absorbs UV light, up to 360 nm, (ii) its intersystem crossing quantum yield (ϕ_{ISC}) is near 1, (iii) the energy of its nπ^* lowest triplet excited state (E_T) is ca. 290 kJ mol^{-1}, and (iv) it produces singlet oxygen ($^1O_2$) with a quantum yield (ϕ_Δ) of ca. 0.3. When these properties of BP are compared with those of riboflavin, a well-known DNA photosensitizer, the main difference is related to the much lower triplet energy value of the latter (ca. 200 kJ mol^{-1}). Accordingly, excited riboflavin is a markedly weaker oxidizing agent and is unable to act as donor in triplet–triplet energy transfer to thymine.

Electron transfer, hydrogen abstraction, and singlet oxygen reactions have been discussed centering attention on guanine, since this is the nucleobase with the lowest oxidation potential. Among oxidative processes, electron transfer is the predominating pathway. Conversely, triplet–triplet energy transfer occurs mainly from ^{3}BP* to thymine, the base with the lowest lying triplet state in DNA. This process results in the formation of cyclobutane pyrimidine dimers, although it competes with the Paternò–Büchi reaction in nucleobases or nucleosides, giving rise to oxetanes as a result of crossed cycloadditions.

In summary, we have presented key insight into the diverse mechanistic pathways of the biologically relevant DNA modifications photosensitized by BP. On the basis of the accumulated experimental data, this chromophore shows potential as a probe for the investigation of electron and triplet energy transport in DNA. The introduction of a chiral center, as in KP, provides a useful tool to examine stereochemical aspects of the involved processes.

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BIOGRAPHICAL INFORMATION

M. Consuelo Cuquerella obtained her Ph.D. from the Technical University of Valencia at the Institute of Chemical Technology

(UPV-CSIC) studying the oxidative DNA damage induced by fluoroquinolones. In June 2004, she moved to the Department of Physics of the University of Liverpool as a postdoctoral fellow. Back to Spain in 2007, she was granted a Juan de la Cierva contract at the University of Valencia. Since 2009, she has been a member of Prof. Miranda's group as a JAE-Doc researcher and her work is mainly focused in the investigation of photoinduced damage to DNA.

Virginie Lhiaubet-Vallet graduated in 1997 and obtained her PhD degree in 2001 from the University Paul Sabatier (France), working on DNA damage photoinduced by nonsteroidal anti-inflammatory drugs. She then joined the group of Prof. M. A. Miranda at the Institute of Chemical Technology (UPV-CSIC) as a postdoctoral researcher benefiting from an Individual Marie Curie European Fellowship. Virginie Lhiaubet-Vallet received the Young Investigator Award from the European Society for Photobiology in 2007. Since 2008, she has been a "Ramón y Cajal" Researcher from Spanish National Research Council at the Institute of Chemical Technology.

Jean Cadet received his Ph.D. in chemistry from the University of Grenoble in 1973 and has been the Head of Laboratory of "Lésions des Acides Nucléiques" at the French Atomic Energy Commission, CEA/Grenoble, until 2001. He is currently Scientific Adviser at CEA/Grenoble and Adjunct Professor at University of Sherbrooke. He is involved in research activities on various aspects of the chemistry and biochemistry of oxidatively generated and photoinduced damage to DNA (mechanisms of reactions, measurement in cells, assessment of biological features, such as substrate specificity of DNA repair enzymes, and mutagenesis of base lesions). He has received several awards including Research Award from American Society for Photobiology, the medal of Excellence from European Society for Photobiology, the Charles Dhéré Award, and Berthelot Medal from the French Academy of Sciences.

Miguel A. Miranda is Professor of Organic Chemistry at the Polytechnical University of Valencia and Head of the Institute of Chemical Technology (UPV-CSIC). He was Associate Professor at the University of Valencia before accepting his present position in 1990. His research interests are mainly focused on photochemistry and photobiology. Miguel A. Miranda has received the Honda-Fujishima Award of the Japanese Photochemistry Association, the Organic Chemistry Award of the Spanish Royal Society of Chemistry, and the Theodor Förster Award of the German Chemical Society and the Bunsen Society of Physical Chemistry. He has been the President of the European Society for Photobiology from 2009 to 2011.

FOOTNOTES

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The authors declare no competing financial interest.

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