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Photogeneration of 2-Deoxyribonolactone in Benzophenone−Purine Dyads. Formation of Ketyl−C1′ Biradicals

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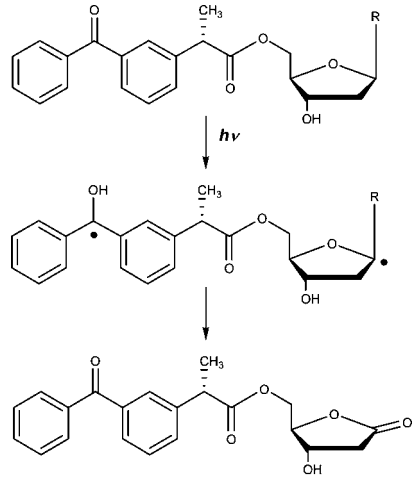
ABSTRACT



R = adenine, guanine or 8-oxoadenine

Photolysis of the title dyads under aerobic conditions leads to a 2-deoxyribonolactone derivative. Laser flash photolysis reveals that the process occurs from the short-lived benzophenone-like triplet excited state. A mechanism involving intramolecular electron transfer with the purine bases (adenine, guanine, or 8-oxoadenine) as donors is proposed.

Chemical modification of DNA produces dramatic consequences in the normal function of living systems, due to alterations of the genetic material during cell replication.[1] Oxidatively generated damage to DNA may be induced by a number of agents, most frequently free radicals, reactive oxygen species (ROS), UV light, or ionizing radiations.[2] The main target units are the nucleobases and the sugar moiety. Loss of a base is one of the most frequent lesions produced in DNA and has been shown to be highly mutagenic.[3] Formation of abasic sites modifies the spatial conformation on the double helix and interferes with the correct function

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of the repair enzyme machinery.[4] These abasic sites may arise from generation of a C1′ radical and subsequent formation of a 2-deoxyribonolactone (dL), and abstraction of the H1′ hydrogen at the anomeric carbon by an appropriate agent is the common initiation step. Sugiyama reported that irradiation of 5-halouracil inserted in DNA generates dL in the adjacent adenine, by a mechanism involving initial formation of a C1′ sugar radical.[5] On the other hand, Chatgilialoglu, Newcomb, and co-workers have found that reaction of the 2′-deoxyuridin-1′-yl radical with molecular oxygen leads to dL as the final product.[6]

Even though it is generally agreed that DNA oxidation primarily occurs at guanine sites, Schuster, Majima, and others have found that the adenine radical cation may also be involved in charge-migration processes.[7] In spite of the importance of this nucleobase, comparatively little information is available on its oxidation chemistry and specifically on the generation of dL by photosensitization.

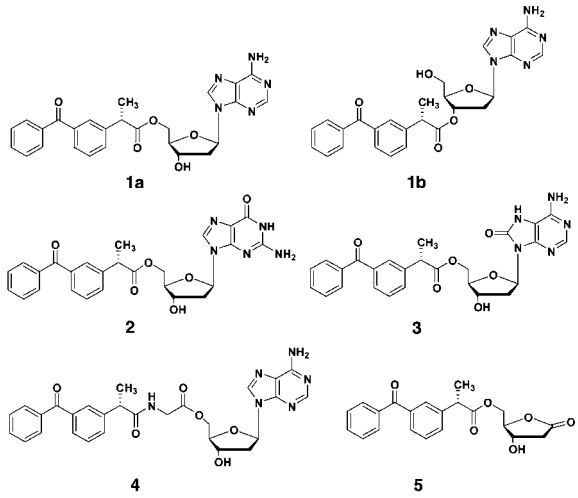
With this background, the aim of the present work is the use of several benzophenone (BP)−purine dyads (Scheme 1) to perform a mechanistic study on the photosensitized oxidation of adenine, with special emphasis on the possible formation of dL as a lesion resulting from this type of process.[8] In these dyads, the BP unit is provided by *S*-ketoprofen (KP), a well-known photosensitizing drug, which has been widely employed for mechanistic studies on nucleosides.[9] The structural variations were designed to evaluate the influence of the *cisoid* vs *transoid* spatial arrangement (**1a** vs **1b**), the base reactivity (series **1a**, **2**, **3**), and the length of the spacer (**1a** vs **4**).[10]

The dyads were synthesized by condensation of KP or its glycine derivative (KPGly) with 2′-deoxyadenosine (dA), 2′-deoxyguanosine (dG), or 8-oxo-7,8-dihydro-2′-deoxyadenosine (8-oxodA), using a carbodiimide as an activating agent. Further details can be found in the Supporting Information section (pages S2−S12).

Upon UVA irradiation (350 nm) of 5′-KP-dA, 5′-KP-dG, and 5′-KP-8-oxodA under oxygen in acetonitrile−water (4:1 v/v), the same 2-deoxyribonolactone (**5**) was isolated in all cases (see spectra in pages S13−S16).

After confirming photochemical formation of the dL lesion in the dyads, time-resolved studies were performed. Selective excitation of the BP chromophore was achieved at 308 and 355 nm. As expected, the typical triplet−triplet BP absorption maximum at $\lambda = 530$ nm was detected in acetonitrile solutions (inset of Figure 1). The triplet lifetimes (τ_T) were strongly dependent on the position in which the KP unit was linked to the sugar moiety: for 3′-KP-dA, the decay was similar to that of isolated ketoprofen, whereas for 5′-KP-dA, the decay was much faster and occurred in the submicrosecond time scale (Figure 1). This can be safely attributed to a more effective interaction between the chromophores

Scheme 1. Structures of the Investigated Compounds



1a. 5′-KP-dA, **1b.** 3′-KP-dA, **2.** 5′-KP-dG, **3.** 5′-KP-8-oxodA, **4.** 5′-KPGly-dA, **5.** 5-KP-dL.

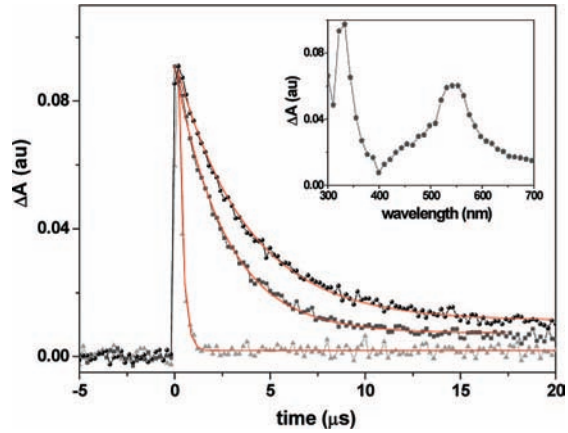


Figure 1. Triplet decays of *S*-ketoprofen (—●—), 3′-KP-dA (—■—), and 5′-KP-dA (—▲—) monitored at 530 nm following laser excitation (308 nm), in acetonitrile solutions (1×10^{-4} M). Inset: Triplet−triplet transient absorption spectrum for 5′-KP-dA recorded following laser excitation (308 nm) in deareated acetonitrile, 30 ns after the laser pulse.

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in the *cisoid* dyad, as previously observed for thymine analogues.[10] The same trend was observed for 5′-KP-dG and 5′-KP-8-oxodA, whose decays also occurred in the submicrosecond range.

Thus, in the 5′-dyads, the intramolecular interaction between KP and the purine base resulted in a significant quenching of the excited BP-like triplet state. This could in principle be associated with an electron transfer process; however, the charge separated species were not detected under the employed experimental conditions, probably due to a very fast back electron transfer. To stabilize such species and to enhance the prospects of detecting the purported intermediates, water was added to the system. As a matter of fact, laser flash photolysis (LFP) of acetonitrile−water solutions (4:1 v/v) of 5′-KP-dA, 5′-KP-dG, and 5′-KP-8-oxodA yielded new transients with maxima at 550 nm (Figure 2), assigned to the characteristic BP−ketyl radical

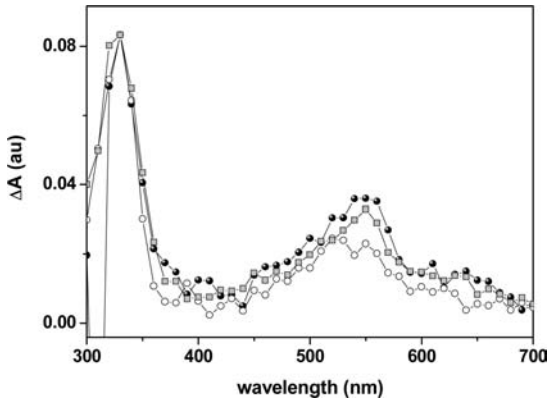

Figure 2. Transient absorption spectra of 5′-KP-dA (—○—), 5′-KP-dG (—●—), and 5′-KP-8-oxodA (—■—) in acetonitrile−water solutions, 30 ns after the 308 nm laser pulse.

moieties.[11] At very early stages (<30 ns), the KP triplet excited states were also detected, although the features of the BP−ketyl radicals were already present. Expectedly, the use of mixed organic−aqueous media also increased the electron transfer efficiency, thus shortening the triplet lifetimes.

Hence, to obtain an accurate measurement of the τ_T values, the naphthalene (NP) quenching method developed by Scaiano was employed.[12] When increasing amounts of NP were added to the dyads, deactivation of the KP triplet excited state (selectively excited at 355 nm) was accompanied by generation of the NP triplet at 425 nm. A double reciprocal plot of the absorbance increment (ΔA_{425}) against NP concentration (Supporting Information, see pages S17−S18) provided the τ_T values upon application of eq 1

$$1/\Delta A_{425} = \alpha + (\alpha/k_q\tau_T)[\mathrm{NP}]^{-1} \qquad (1)$$

where k_q = intermolecular rate constant for quenching of KP by NP (4×10^9 M^{-1} s^{-1}), ΔA_{425} = end of pulse absorbance at 425 nm (maximum naphthalene triplet formation), and α = constant.

Operating in this way, the values determined for the KP triplet lifetimes in the dyads were 30 ns for 5′-KP-dA, 9 ns for 5′-KP-dG, and 12 ns for 5′-KP-8-oxodA. On the basis of these results, estimation of the involved rate constants was made using eq 2

$$k_{intra} = (1/\tau_T - 1/\tau_0) \qquad (2)$$

where k_{intra} = intramolecular quenching rate constant, τ_0 = triplet lifetime of free KP, and τ_T = triplet lifetime in the dyads. The values obtained (3.3×10^7 s^{-1}, 1.1×10^8 s^{-1}, and 8.3×10^7 s^{-1} for 5′-KP-dA, 5′-KP-dG, and 5′-KP-8-oxodA) are in agreement with the expectations for an electron transfer mechanism. Thus, to demonstrate the viability of the process, the free energy changes (ΔG, kJ/mol) were calculated using the Rehm−Weller eq 3

$$\Delta G = 96.5[E^0(D^{+\bullet}/D) - E^0(A/A^{-\bullet})] - \Delta E_{oo} \qquad (3)$$

where $E^0(D^{+\bullet}/D)$ is the one-electron oxidation potential of the nucleobases; $E^0(A/A^{-\bullet})$ is the one-electron reduction potential of the photosensitizer, and ΔE_{oo} is the triplet excited-state energy (KP or BP), determined by spectroscopic measurements. In this context, negative ΔG increments for the intermolecular process in related D/A systems have been reported by Lhiaubet et al.[13] for dA ($\Delta G = -59$ kJ/mol, $E^0 = 1.18$ V vs SCE) and dG ($\Delta G = -71$ kJ/mol, $E^0 = 1.05$ V vs SCE) nucleosides, when *S*-KP was used as an acceptor ($E^0 = -1.24$ V vs SCE, $\Delta E_{oo} = 292.6$ kJ/mol) in aqueous media. These findings are consistent with our results for dyads **1a**, **2**, and **3** by laser flash photolysis. Even though no E^0 values were available for **3**, it can be assumed that the relative free energy changes would be dG > 8-oxodA > dA, in accordance with the nucleoside reactivities.

For the same donor−acceptor pair, the electron transfer process should be sensitive to the length of the spacer. To evaluate the influence of this factor, 5′-KPGly-dA was studied by time-resolved experiments under the same conditions. The transient absorption spectrum displayed the two typical T−T bands with maxima at 330 and 530 nm (see pages S18−S19 in Supporting Information). Its lifetime (0.4 μs) was much longer than that of 5′-KP-dA (0.03 μs), and the rate constant determined by application of eq 2 was accordingly lower (2.2×10^6 s^{-1}). This constitutes a further support for the proposed electron transfer mechanism. Scheme 2 summarizes the sequential steps leading from the dyads to the final 2-deoxyribonolactone product. Here, deprotonation of the purine radical cation at C1′ could be achieved by the BP-like radical anion. Assistance of the aqueous medium seems to be esential, as otherwise the ketyl radical could not reach C1′. The $(KP)^{\bullet-}$ absorption (630 nm) was not observed in the nanosecond time scale. However, the corresponding $(KPH)^{\bullet}$ band (550 nm) was clearly detected in the LFP experiments. Previously, Cadet and co-workers have isolated dL after UVA irradiation of 2′-deoxyguanosine (dG) in the presence of BP, and its formation has been attributed to photochemical electron transfer, followed by deprotonation of the radical cation at the C1′ position. The

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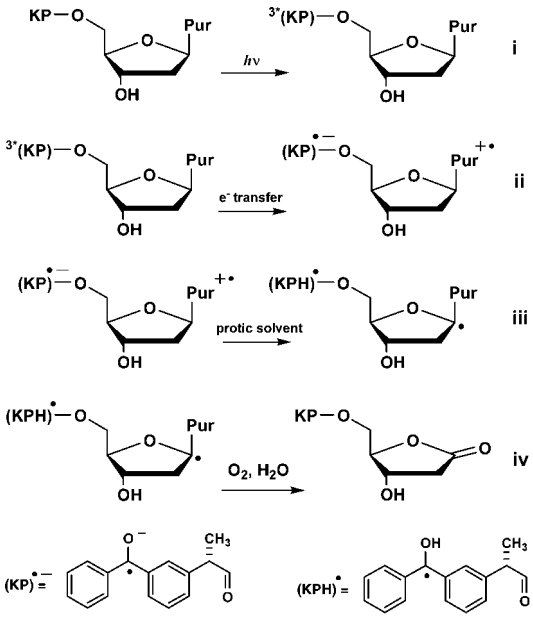
Scheme 2. Summary of the Sequential Steps Leading from the Dyads to 5′-KP-dL



BP-sensitized irradiation of a dinucleotide (dTdG) leads to formation of dL at the dG site. In the absence of BP or in the presence of a phthalocyanine derivative as a type II photosensitizer, no significant dL formation is observed.[14] In addition, 2-deoxyribonolactone has been identified among the 7-methylpyrido[3,4-c]psoralen photosensitized degradation products of 2′-deoxyadenosine in the solid state.[15]

Estimation of the ketyl−C1′ biradical quantum yields in the dyads was done by determining the maximum transient absorbance of optically matched acetonitrile−water solutions ($A = 0.25$) at 570 nm, after 308 nm LFP excitation. Using KP as a standard for comparison ($\Phi = 1$), the obtained results ($\Phi = 0.82$ for 5′-KP-dA, $\Phi = 0.95$ for 5′-KP-dG, and $\Phi = 0.88$ for 5′-KP-8-oxodA) were in agreement with the relative oxidizability of the bases. The biradical lifetimes were determined from the first-order decays and found to be 0.17 μs for 5′-KP-dA, 0.21 μs for 5′-KP-dG, and 0.30 μs for 5′−8-oxo-dA: they were remarkably shorter than those previously reported for the KP-derived ketyl radical (>20 μs) and for the nucleoside C1′ radicals (typically 10−20 μs).[16,6] Biradicals are known to live in the submicrosecond range, and their deactivation proceeds through a complex combination of different processes, including intersystem crossing to the singlet biradicals, back hydrogen transfer, radical coupling, etc.[17]

Although direct observation of purine-derived C1′ radicals as transient species has not been reported yet, Chatgilialoglu, Newcomb, and co-workers have performed time-resolved studies on pyrimidine analogues.[6] Thus, generation of ketyl−C1′ biradicals is potentially interesting as a model for kinetic measurements on C1′ radical reactivity with oxygen or reducing agents, which is relevant in connection with the oxidation and repair of this lesion. In the present work, these hardly detectable sugar radicals are made visible through the KP-reduced moiety, which absorbs in a longer wavelength region and is therefore more convenient for experimental purposes.

In summary, a 2-deoxyribonolactone is formed by photolysis of KP-tethered purine nucleosides, through the generation of C1′−ketyl biradicals. Transient absorption spectroscopy supports an intramolecular electron-transfer mechanism. These results are potentially applicable for direct kinetic measurements on C1′ sugar radical reactivity.

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Supporting Information Available: Experimental procedures of synthesis, photolysis, characters of compounds, and LFP data. This material is available free of charge via the Internet at http://pubs.acs.org.

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