

Photosensitized oxidation of purine nucleotides by pterin in aqueous solutions

INTRODUCTION

In this doctoral thesis we investigated the photosensitization of 2'-deoxyadenosine 5'-monophosphate (dAMP) and 2'-deoxyguanosine 5'-monophosphate (dGMP) by pterin (PT) in aqueous solution under UV-A irradiation.

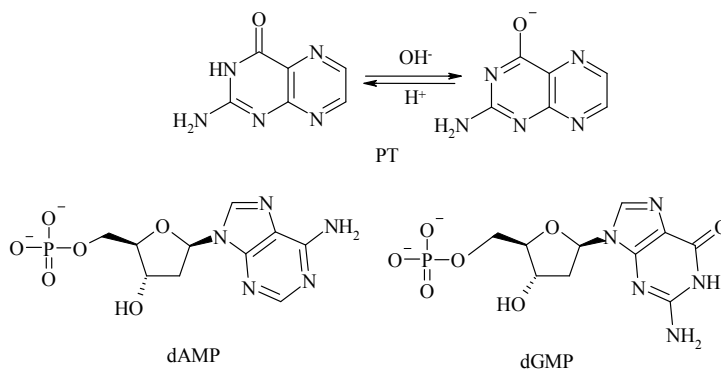


Figure 1: Molecular structure of PT, dGMP and dAMP

Solar radiation induces modifications to genomic DNA and is implicated in the induction of human skin cancers. UV radiation is the most mutagenic and carcinogenic component of the solar radiation. UV-B radiation (280-320 nm) damages DNA through the direct excitation of the nucleobases. On the other hand, although nucleobases absorb very weakly above 320 nm, UV-A radiation (320-400 nm) may damage DNA through photosensitized reactions.¹ This indirect action is mediated by a photosensitizer (endogenous or exogenous) which is excited by the UV-A radiation.

The chemical changes in DNA and its components resulting from photosensitized reactions can take place through different mechanisms. It has been demonstrated that energy transfer from the triplet state of the photosensitizer to pyrimidine bases leads to the formation of pyrimidine dimers.¹ Photosensitized oxidations also contribute to DNA damage induced by UV-A radiation. These processes involve the generation of radicals (type I), *e.g.*, via electron transfer or hydrogen abstraction, and/or the production of singlet molecular oxygen (¹O₂) (type II).²

Pterins, heterocyclic compounds widespread in biological systems, are derived from 2-aminopteridin-4(1*H*)-one or pterin (PT) (Fig. 1). Several pterin derivatives participate in important biological processes such as the synthesis of amino acids and nucleobases, nitric oxide metabolism and the activation of cell-mediated immune responses. Pterins behave as weak acids in aqueous solutions, the dominant equilibrium at pH > 5 involving an amide group (acid form) and a phenolate group (basic form) (Fig.1, p*K*_a = 7.9 for PT). The participation of pterins in photobiological processes has been suggested or demonstrated in the past decade, and interest in the photochemistry and photophysics of these compounds has subsequently increased. Under UV-A excitation, these biomolecules can fluoresce, undergo photooxidation to produce different photoproducts and generate reactive oxygen

species such as $^1\text{O}_2$.³ Interestingly, some pterin derivatives (*e.g.* biopterin, 6-formylpterin, 6-carboxypterin) accumulate in the skin of patients affected by vitiligo, a depigmentation disorder, where protection against UV radiation fails due to the lack of melanin.⁴

The capability of pterins to photoinduce damage to DNA was demonstrated for the first time in 1997.⁵ Taking into account indirect evidence, the mechanism involved in this process was proposed to be an electron transfer with the subsequent formation of the guanine radical cation and a pterin radical anion. Later studies provided additional evidence on the photosensitizing capability of pterins.^{6,7} On the other hand, in a very recent work, photosensitization *via* $^1\text{O}_2$ has been reported as the main mechanism responsible for the photoinduced cleavage of plasmid DNA by pterins.⁸

SUMMARY OF RESULTS

1-The nucleotides dGMP and dAMP deactivate $^1\text{O}_2$ with different efficiency and through different mechanisms.

The quenching of $^1\text{O}_2$ by dGMP is mainly a chemical process. Within the experimental error, the values of the rate constant of $^1\text{O}_2$ total quenching by dGMP (k_t (1.7 ± 0.1) $\times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ and (8.5 ± 0.6) $\times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ in acidic and alkaline media, respectively) are similar to the values of the rate constant of the chemical reaction between $^1\text{O}_2$ and dGMP in the corresponding media (k_r (1.7 ± 0.3) $\times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ and (9.6 ± 0.8) $\times 10^7 \text{ M}^{-1} \text{ s}^{-1}$, respectively). It is noteworthy that the reactivity of dGMP toward $^1\text{O}_2$ is much higher in alkaline media. This fact can be explained on the basis of charge effects as a consequence of the different acid-base equilibria of the nucleotides in H_2O . At pH 10.5, the guanine moiety is deprotonated at its lactam group. Since the attack of $^1\text{O}_2$ takes place onto the guanine moiety, its deprotonation must be responsible for the high increase of the k_r value with the pH. On the other hand, in the case of dAMP, the physical quenching predominates and the contribution of chemical reaction is negligible ($k_t^{\text{dAMP}} = (4.1 \pm 0.4) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, $k_r^{\text{dAMP}} = (8 \pm 3) \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$).

2-Pterin is able to photosensitize dAMP and dGMP in aqueous solutions under UV-A irradiation. The rate of the reaction and the mechanisms depends on the nucleotide and the value of pH.

When an aerated solution containing dAMP and the acid form of PT was exposed to UV-A radiation dAMP was consumed, whereas the photosensitizer (PT) concentration did not change significantly. During this process, O_2 was consumed and H_2O_2 was generated. A significant increase in the consumption of dAMP was observed when superoxide dismutase was present in the solution. This enzyme catalyzes the conversion of superoxide anion ($\text{O}_2^{\cdot-}$) into H_2O_2 and O_2 . This result suggests that elimination of $\text{O}_2^{\cdot-}$ inhibits a step that prevents the photoinduced oxidation of dAMP. Moreover, it was demonstrated that $^1\text{O}_2$ does not participate in the photosensitization of dAMP by PT.

Two products formed during the photosensitization of dAMP were identified by ESI mass analysis: 8-oxo-7,8-dihydro-2'-deoxyadenosine 5'-monophosphate (8-oxo-dAMP) and a "product 2" (Fig. 2). 8-Oxo-7,8-dihydro-2'-deoxyadenosine (8-oxo-dAdo) has been proposed as a product of the photosensitized oxidation of 2'-deoxyadenosine (dAdo) in DNA *via* a type I mechanism.⁹ Therefore, the results support the hypothesis of an electron transfer from dAMP to excited PT. The MS/MS spectra of "product 2" suggests that a $-OP(=O)(OH)O-$ bridge has been formed between the phosphate and the C8 of the adenine moiety (Fig. 2).

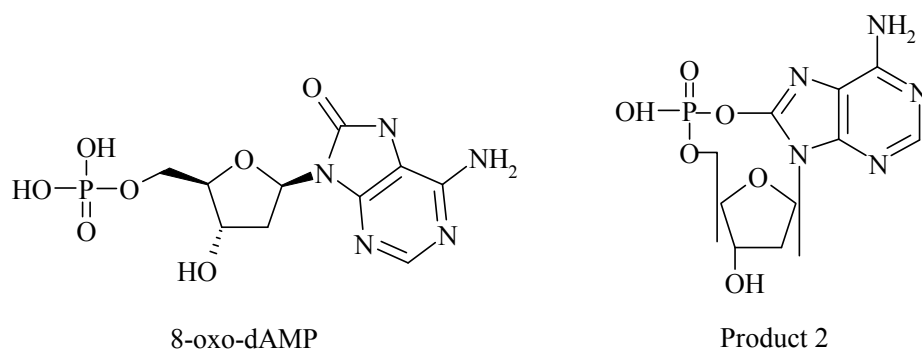


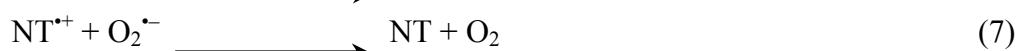
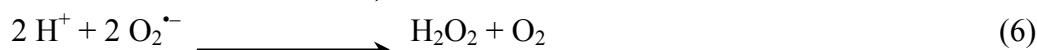
Figure 2: Molecular structure of oxidation products of dAMP.

In contrast, no evidence of a photochemical reaction induced by the basic form of PT was observed. No spectral changes and no decrease of the dAMP concentration were detected in experiments carried out at pH = 10.5. Photosensitization was not observed even at relatively high concentration of dAMP (1 mM) and long irradiation times (more than 2 hours). Probably, the process of electron transfer from the nucleotide towards PT is less efficient for the alkaline form than for acid form because PT is negatively charged at 10.5. Another factor to consider is the dismutation of $O_2^{\bullet-}$ in H_2O_2 and O_2 and their dependency with the pH. The $O_2^{\bullet-}$ is consumed mainly through its protonated form HO_2^{\bullet} (pKa = 4.85). Therefore, in the acidic media the lifetime of $O_2^{\bullet-}$ is smaller than in the alkaline media. Then at pH 10.5 the reaction between $dAMP^{++}$ and $O_2^{\bullet-}$ could contribute significantly to avoid the consumption of dAMP.

When aerated solutions containing dGMP and PT at pH 5.5 are exposed to UV-A radiation, dGMP is consumed, whereas the photosensitizer (PT) concentration does not change significantly. During this process, O_2 is consumed, H_2O_2 and highly polar compounds are generated. Additionally, $O_2^{\bullet-}$ acts as an inhibitor of the process. The role of 1O_2 in the oxidation of dGMP photosensitized by PT was evaluated from kinetic calculations taking into account the values obtained for k_T (*vide supra*) and from comparative photolysis experiments performed in H_2O and D_2O . Results showed that the chemical reaction between dGMP and 1O_2 does not contribute significantly to the photosensitized oxidation of dGMP by the acid form of PT (pH 5.5), thus suggesting the participation of a type I mechanism under these pH conditions. Direct evidence of electron transfer between dGMP and excited PT was obtained in acidic media by laser flash photolysis experiments showing the presence of the

dGMP radical after excitation of PT at 355 nm. The formation of such a radical occurs within a time window of a few microseconds, which indicates the participation of the PT triplet state in the process. Kinetic calculations showed that despite dGMP is able to deactivate singlet excited state of PT ($^1\text{PT}^*$), this is not involved in the electron transfer. This result suggests, that the PT triplet state ($^3\text{PT}^*$) is in charge of this process. This hypothesis was finally confirmed comparing experiments of quenching of the luminescence of $^1\text{O}_2$ by dGMP in which reference photosensitizers and PT were used. The interaction between the triplet excited state ($^3\text{PT}^*$) and dGMP was confirmed by analysis of Stern-Volmer.

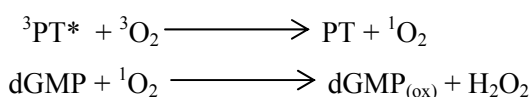
Taking into account the results obtained with dAMP and dGMP, the following general mechanism can be proposed for the oxidation of purine nucleotides photoinduced (NT) by PT in acidic media:



After excitation of PT and formation of its triplet excited state, $^3\text{PT}^*$ (Reactions 1 and 2), electron transfer between the nucleotide (NT) and $^3\text{PT}^*$ leads to the formation of the corresponding radical ions, $\text{PT}^{\bullet-}$ and $\text{NT}^{\bullet+}$ (Reaction 3). In the following step, the radical ions may recombine (Reaction 4), which explains the absence of substrate consumption under anaerobic conditions. Alternatively, the electron transfer from $\text{PT}^{\bullet-}$ to O_2 regenerates the sensitizer and forms $\text{O}_2^{\bullet-}$ (Reaction 5). This radical disproportionates with its conjugated acid HO_2^{\bullet} to form H_2O_2 (summarized by Reaction 6) or react with $\text{NT}^{\bullet+}$ to regenerate the substrate (Reaction 7). Finally a group of processes, represented schematically by Reaction 8 and that include the reactions of $\text{NT}^{\bullet+}$ and its deprotonated form ($\text{NT}(-\text{H})^{\bullet}$) with O_2 and H_2O , leads to the oxidation of the nucleotide and consumption of O_2 .¹⁰

The nucleotide dGMP, unlike dAMP, is photosensitized by PT in alkaline media. During the reaction dGMP and O_2 are consumed, the PT concentration remains constant and H_2O_2 and several polar products of the oxidation of the nucleotide are produced. In this case the kinetic calculations and the experiments carried out in D_2O showed that photooxidation takes place mainly *via* $^1\text{O}_2$. Therefore, the mechanism proposed for alkaline media is:





Type I and type II mechanisms of the oxidation of dGMP photosensitized by PT are competitive and contribute in different proportions depending on the pH. In alkaline media, where the quantum yield of ${}^1\text{O}_2$ production by PT (Φ_{Δ}) and the rate constant of the chemical reaction between dGMP and ${}^1\text{O}_2$ (k_r) are higher than those in acidic media ($\Phi_{\Delta}^{10.5}=0.30$; $\Phi_{\Delta}^{5.5}=0.18$; $k_r^{10.5} = (9.6 \pm 0.8) \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$; $k_r^{5.5} = (1.7 \pm 0.3) \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$), the main mechanism involves ${}^1\text{O}_2$ as the reactive intermediate. On the other hand, under acidic conditions, where the reaction with ${}^1\text{O}_2$ is much slower and the initial electron transfer is likely to be more efficient, the type I mechanism is the main pathway. Since this is the situation at physiological pH, it can be inferred that electron transfer should be the main mechanism responsible for oxidation of nucleotides photosensitized by PT in biological systems.

3-Deoxynucleotides are able to quench singlet excited states of pterin derivatives

Steady-state and time-resolved studies of the fluorescence of four aromatic unconjugated pterins (PT, 6-(hydroxymethyl)pterin, 6-methylpterin, and 6,7-dimethylpterin) in aqueous solutions in the presence of different nucleotides (dGMP, dAMP and 2'-deoxycytosine 5'-monophosphate (dCMP)) were performed using the single-photon counting technique. The singlet excited states of acid forms of pterins are deactivated by purine nucleotides (dGMP and dAMP) *via* a combination of dynamic and static processes. The efficiency of the dynamic quenching is high, independently of the nature of the purine base of the nucleotide and of the chemical structure of the substituents linked to the pterin moiety. Analysis of the static quenching indicates that ground-state association between pterins and purine nucleotides takes place, but the formation of the corresponding complexes is significant only at relatively high reactant concentrations. The quenching of the fluorescence of acid forms of pterin derivatives by dCMP, a pyrimidine nucleotide, is slightly less efficient than the quenching by purine nucleotides and is purely dynamic. In alkaline media, the fluorescence quenching is much less efficient than in acidic media, the deactivation by purine nucleotides being purely dynamic, whereas quenching by dCMP is negligible.

- 1 J.-L. Ravanat, T. Douki and J. Cadet, *J. Photochem. Photobiol., B*, 2001, **63**, 88–102.
- 2 C. S. Foote, *Photochem. Photobiol.*, 1991, **54**, 659
- 3 C. Lorente and A. H. Thomas, *Acc. Chem. Res.*, 2006, **39**, 395–402
- 4 K. U. Schallreuter, J. M. Wood, M. R. Pittelkow, M. Gutlich, K. R. Lemke, W. Rodl, N. N. Swanson, K. Hitzemann and I. Ziegler, *Science*, 1994, **263**, 1444–1446.
- 5 K. Ito and S. Kawanishi, *Biochemistry*, 1997, **36**, 1774–1781.
- 6 C. Lorente, A. H. Thomas, L. S. Villata, D. Hozbor, A. Lagares and A. L. Capparelli, *Pteridines*, 2000, **11**, 100–105.
- 7 K. Hirakawa, H. Suzuki, S. Oikawa and S. Kawanishi, *Arch. Biochem. Biophys.*, 2003, **410**, 261–268.
- 8 C. Martí, O. Jürgens, O. Cuenca, M. Casals and S. Nonell, *J. Photochem. Photobiol., A*, 1996, **97**, 11–18.
- 9 T. Douki and J. Cadet, *Int. J. Radiat. Biol.*, 1999, **75**, 571–581
- 10 J. Cadet, M. Berger, T. Douki, B. Morin, S. Raoul, J.-L. Ravanat and S. Spinelli *Biol. Chem.* 1997, **378**, 1275–1286