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N-Hydroxypyridine-2(1*H*)-thione: Not a Selective Generator of Hydroxyl Radicals in Aqueous Solution

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The study of radical-induced damage to living systems is a topic of great interest in biology and medicine.[1,2] Experimentally, one is confronted with the problem of how to specifically generate radicals in order to investigate their biological effects. Photosensitization is often complicated by the production of long-lived excited states of the photosensitizer and subsequent formation of singlet molecular oxygen, which can also contribute to the resulting damage. In the search for mediators of purely radical processes, we have recently shown ester derivatives of *N*-hydroxypyridine-2(1*H*)-thione to be efficient and selective free radical generators.[3]

The various physical and chemical methods developed for producing the hydroxyl radical ($^{\bullet}$OH), a species known to be involved in many important beneficial or deleterious biological processes, are not fully satisfactory. For example, in the Fenton reaction, the formation of other oxidizing agents concomitant to $^{\bullet}$OH[2] is a major drawback for an unambiguous identification of the species responsible for the damage. Recently, the photoexcitation of *N*-hydroxypyridine-2(1*H*)-thione (N-HPT) has been proposed as an improved alternative approach for formation of $^{\bullet}$OH.[4−6] UV irradiation of N-HPT (Figure 1) in organic solvents[5,7,8] or in aqueous media[6,8,9] induces a homolytic scission of the nitrogen−oxygen bond, resulting in the formation of the 2-pyridylthiyl (PyS$^{\bullet}$) and hydroxyl radicals. The production of $^{\bullet}$OH from N-HPT has been demonstrated by the formation of $^{\bullet}$OH−spin trap adducts in electron paramagnetic resonance experiments[10] and by chemical assays.[6] It was concluded that facile photogeneration of a continuous flux of $^{\bullet}$OH under mild conditions makes this an attractive approach for the selective study of $^{\bullet}$OH reactions in biologically relevant systems.[6] However, this conclusion seems premature since the detection of $^{\bullet}$OH does not necessarily mean that the photoactivation of N-HPT is a clean and unambiguous source of $^{\bullet}$OH, as other metastable species may also be produced and contribute to the effects observed.

We report here pertinent results of our investigation of the primary photochemistry of N-HPT in aqueous media by laser flash photolysis.[11] They reveal that, contrary to the growing belief, the biological effects of this compound cannot be considered as unequivocally resulting from reactions of $^{\bullet}$OH since (1) under UV irradiation in aqueous buffered solution (pH = 7) N-HPT also undergoes photoionization and (2) PyS$^{\bullet}$ was found to be potentially reactive toward biological molecules.

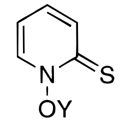
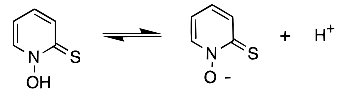


Figure 1. Chemical structure of *N*-hydroxypyridine-2(1*H*)-thione. For the free form of N-HPT, Y = H; for the sodium salt, Y = Na.

Laser flash photolysis experiments[12,13] utilized the frequency-tripled output of a Nd:YAG laser (λ_{exc} = 355 nm, 10 ns pulse duration) or a XeCl excimer laser (λ_{exc} = 308 nm, 8 ns pulse duration) as excitation source to study the primary photochemistry of N-HPT[14] in buffer (pH = 7).[15] A flow system was used in order to irradiate a fresh sample volume with each excitation pulse. N-HPT is potentially tautomeric; it can exist under the thione form and the corresponding thiol tautomer. In aqueous media, the predominance of the thione over the thiol structure has been demonstrated.[16] In addition, N-HPT exhibits an acid/base equilibrium (pK_a = 4.67[16]) such that, at pH = 7, it is mainly present as the anionic form of the thione tautomer.



(1)

The transient absorption spectra recorded after pulsed irradiation of a nitrogen-purged solution of N-HPT using either 308 or 355 nm excitation present similar features. In comparison to thiohydroxamic esters in organic solvents,[3] where N−O bond cleavage is the only primary photoprocess, the photochemistry of N-HPT in aqueous media appears more complex. Figure 2 shows the time-dependent transient absorption spectrum recorded following laser excitation. At the earliest times (≤1 μs), a large, broad band with a maximum around 720 nm is observed. This absorption, which is quenched in the presence of oxygen or nitrous oxide and has a lifetime of 450 ns under the experimental conditions used to record the spectrum, is assigned to the hydrated electron.[17] In addition, a strong absorption is observed at 375 nm which decays rapidly with a time constant of 130 ns. It probably corresponds to the semioxidized radical of N-HPT, formed on photoionization. The spectrum also presents an instantaneous negative band with a maximum around 335 nm due to ground-state depletion of the starting material. Following decay of the hydrated electron and the species at 375 nm, two absorption bands with maxima at 370 and 460 nm become visible. The species with the absorption maximum at 460 nm (which has a lifetime of 25 μs) was found to be efficiently quenched by *trans,trans*-2,4-hexadien-1-ol but unaffected by the presence of oxygen. This behavior is similar to that observed for PyS$^{\bullet}$ generated by irradiation of the ester derivatives of N-HPT in organic solvents (acetonitrile and benzene).[3] A blue shift of the absorption spectrum of PyS$^{\bullet}$ in water (compared to that in organic solvents where the maximum is situated at 490 nm) is not surprising

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(9) In aqueous solutions, both the sodium salt and the free form of N-HPT were reported to produce $^{\bullet}$OH.

(10) Reszka, K.; Chignell, C. F. *Photochem. Photobiol.* **1995**, *61*, 269−275.

(11) A complete description of the photochemistry of N-HPT and related compounds will be published soon.

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(14) The results reported here are for the sodium salt of N-HPT.

(15) As with numerous hydroxamic acid analogues, *N*-hydroxypyridine-2(1*H*)-thione has the capability to chelate metallic ions, especially Fe^{2+}. Some of its biological properties, such as antifungal and antibacterial activities, are believed to result from this chelating ability. In the preparation of the buffered solution (KH_2PO_4/NaOH, 10 mM, pH = 7), we have used deionized water (*i.e.* free of iron) in order to avoid possible formation of chelates which may modifiy the photoproperties of N-HPT.

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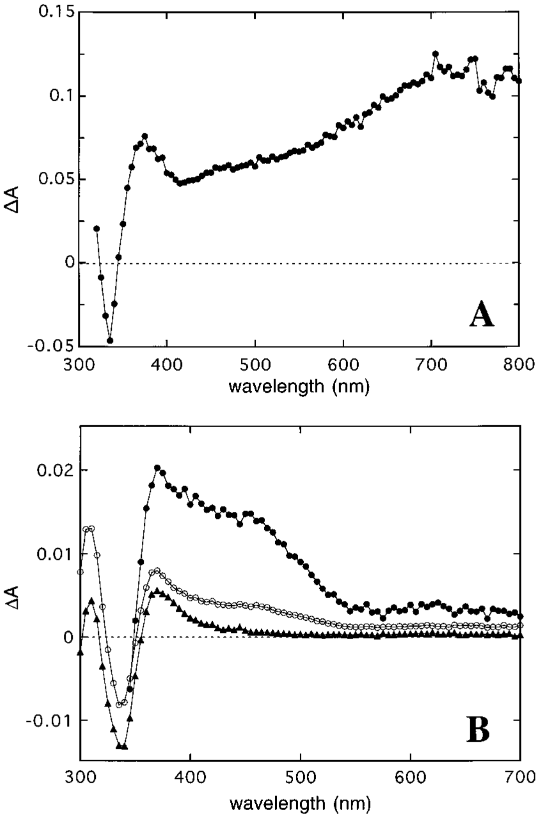

Figure 2. Transient absorption spectra recorded for *N*-hydroxypyridine-2(1*H*)-thione sodium salt in degassed, deionized water (A) : 160 ns (●) and (B) 1.4 μs (●), 10 μs (○) and 140 μs (▲) after laser flash excitation ($\lambda_{\mathrm{exc}} = 308$ nm). The absorbance of the solution at 308 nm was 0.35, corresponding to a ground-state concentration of 160 μM, and the laser energy was 10 mJ/pulse.

since (1) it parallels the blue shift of the absorption spectrum of the ground state of N-HPT (for which the chromophore is the pyridinethione group) and (2) a similar shift was also observed in the case of the closely related benzenethiyl radical.[18,19] The spectral results observed here indicate clearly that, under pulsed irradiation, N-HPT undergoes an instantaneous photoionization as well as another photoprocess leading to the formation of the thiyl radical.

To prove that homolytic cleavage of the nitrogen−oxygen bond is the photoprocess giving rise to PyS$^{\bullet}$, we looked for evidence of concomitant formation of $^{\bullet}$OH. Due to its absorption properties (weak band in the deep UV region of the spectrum[17]), $^{\bullet}$OH cannot be observed directly. In aqueous solutions, various acceptors, such as ionic species, can be used to probe the presence of $^{\bullet}$OH: the reaction of these acceptors with $^{\bullet}$OH leads to the formation of radical ions strongly absorbing light in the visible.[2] Figure 3 shows the transient absorption spectrum recorded after excitation of a deaerated aqueous solution of N-HPT in the presence of potassium thiocyanate (KSCN). This compound is known to react with $^{\bullet}$OH according to reactions 2 and 3 to give (SCN)$_2^{\bullet-}$, which is easily detectable by its absorption around 500 nm.[2] The absorption band corresponding to (SCN)$_2^{\bullet-}$ was not formed when $^{\bullet}$OH scavengers (such as phenol, dimethyl sulfoxide, methanol, and ascorbic acid) were added to the solution. It is worthwhile noting that the intensity of this band increases when nitrous oxide (known to quench the hydrated electron to give $^{\bullet}$OH according to reaction 4) is present in the sample.

$$\mathrm{SCN^- + {}^{\bullet}OH \rightarrow SCN^{\bullet} + OH^-} \tag{2}$$

$$\mathrm{SCN^- + SCN^{\bullet} \rightleftharpoons (SCN)_2^{\bullet-}} \tag{3}$$

$$\mathrm{e^-_{aq} + N_2O + H_2O \rightarrow {}^{\bullet}OH + OH^- + N_2} \tag{4}$$

The quantum yields of photoionization (Φ_{e^-}) and $^{\bullet}$OH formation ($\Phi_{^{\bullet}\mathrm{OH}}$) for N-HPT in deaerated aqueous buffered solution (pH = 7) were determined by comparative actinometry using 2′-acetonaphthone (for which $\Phi_{\mathrm{T}} = 0.84$[20] and $\epsilon_{\mathrm{T}} = 10\,500$ M^{-1}cm^{-1} [21] at 430 nm) and benzophenone (for which $\Phi_{\mathrm{T}} = 1.0$[22] and $\epsilon_{\mathrm{T}} = 7220$ M^{-1} cm^{-1} [23] at 530 nm) in benzene as standards. The intensity dependence of the solvated electron absorbance was measured at 720 nm (where $\epsilon = 19\,000$ M^{-1}cm^{-1} [17]) and found to be linear in the whole range of laser energies used, suggesting that photoionization of N-HPT occurs *via* a monophotonic process. Under conditions where enough potassium thiocyanate is present to quench all the hydroxyl radicals produced, the quantum yield of (SCN)$_2^{\bullet-}$ [24] formation is equivalent to $\Phi_{^{\bullet}\mathrm{OH}}$. Using 308 nm as excitation wavelength, Φ_{e^-} and $\Phi_{^{\bullet}\mathrm{OH}}$ were found to be 0.10 and 0.13, respectively.

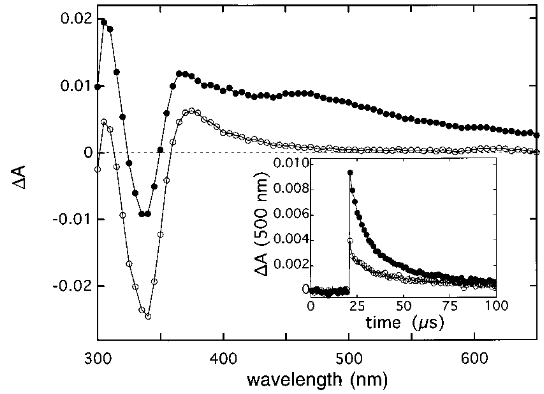

Figure 3. Transient absorption spectra recorded for *N*-hydroxypyridine-2(1*H*)-thione sodium salt in degassed deionized water in the presence of 0.2 M potassium thiocyanate (KSCN), 8 μs (●) and 150 μs (○) after laser flash excitation ($\lambda_{\mathrm{exc}} = 308$ nm). The absorbance of the solution at 308 nm was 0.35, corresponding to a ground-state concentration of 160 μM and the laser energy was 10 mJ/pulse. The inset presents the kinetic signal recorded at 500 nm in the presence (●) and the absence (○) of KSCN.

Furthermore, PyS$^{\bullet}$, which we have already shown to rapidly add to double bonds and to abstract hydrogen,[3] was also found here to be reactive toward biological molecules such as cholesterol ($k_{\mathrm{Q}} = 2 \times 10^6$ M^{-1} s^{-1}) and fatty acids (e.g., $k_{\mathrm{Q}} = 2 \times 10^7$ M^{-1} s^{-1} with linoleic acid).[25]

In summary, laser flash photolysis experiments have demonstrated that UV irradiation ($\lambda_{\mathrm{exc}} = 308$ or 355 nm) of N-HPT in buffer (pH = 7) causes the photoionization of the starting material in addition to the expected homolytic N−O bond cleavage responsible for the generation of hydroxyl and 2-pyridylthiyl radicals. This photoionization, hitherto never observed,[10] obviously compromises the use of N-HPT as an unambiguous source of $^{\bullet}$OH in biologically relevant systems. In addition, the high reactivity of PyS$^{\bullet}$ constitutes another major drawback that cannot be ignored when the photoexcitation of N-HPT is used as a method for studying the effects of $^{\bullet}$OH in biological systems.

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[25] These experiments were carried out under nitrous oxide saturated conditions (to quench the hydrated electron) and in the presence of *tert*-butyl alcohol (to scavenge $^{\bullet}$OH).