Photochemistry and Photobiology, 2005, 81: 325–332

Sensitized Photooxidation of Thyroidal Hormones. Evidence for Heavy Atom Effect on Singlet Molecular Oxygen [$O_2(^1\Delta_g)$]-mediated Photoreactions¶

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Received 27 October 2004; accepted 28 December 2004

ABSTRACT

Thyronine derivatives are essential indicators of thyroid gland diseases in clinical diagnosis and are currently used as standards for developing ordinary biochemical assays. Photooxidation of gland hormones of the thyronine (TN) family and structurally related compounds (TN, 3,5-diiodothyronine,3,3′,5-triiodothyronine and 3,3′,5,5′-tetraiodothyronine or thyroxine) was studied using rose bengal, eosin and perinaphthenone (PN) as dye sensitizers. Tyrosine (Tyr) and two iodinated derivatives (3-iodotyrosine and 3,5-diiodotyrosine) were also included in the study for comparative purposes. Irradiation of aqueous solutions of substrates containing xanthene dyes with visible light triggers a complex series of competitive interactions, which include the triplet excited state of the dye (^{3}Xdye*) and singlet molecular oxygen [$O_2(^1\Delta_g)$]–mediated and superoxide ion–mediated reactions. Rate constants for interaction with the ^{3}Xdye*, attributed to an electron transfer process, are in the order of 10^8–10^9 M^{-1} s^{-1} depending on the dye and the particular substrate. The photosensitization using PN follows a pure Type-II ($O_2(^1\Delta_g)$ mediated) mechanism. The presence of the phenolic group in Tyr, TN and iodinated derivatives dominates the kinetics of photooxidation of these compounds. The reactive rate constants, k_r, and the quotient between reactive and overall rate constants (k_r/k_t values, in the range of 0.7–0.06) behave in an opposite fashion compared with the overall rate constants and oxidation potentials. This apparent inconsistency was interpreted on the basis of an internal heavy atom effect, favoring the intersystem-crossing deactivation route within the encounter complex with the concomitant reduction of effective photooxidation.

INTRODUCTION

There is an increasing interest in the mechanisms for photochemical reactions of compounds of biological relevance because of the possible involvement of such reactions in naturally occurring and biomedical processes (1–4). This is the case with the thyroidal gland hormones (5) and related compounds of the thyronine (TN) family, compounds transparent to environmental natural light, in the following generically named thyroidal hormones (TH) (see Table 1, Ref. 6) formed by TN, 3,5-diiodothyronine (T2), 3,3′,5-triiodothyronine (T3) and 3,3′,5,5′-tetraiodothyronine or thyroxine (T4). These compounds possess the common basic structure of *p*-phenolic ethers (Scheme 1). Photoprocesses involved in sensitized aerobic and anaerobic reactions of phenolic derivatives (P) have been extensively investigated (for reviews on the topic see Refs. 7 and 8). These derivatives (P), in general, are excellent candidates for suffering visible light–mediated sensitized photooxidation in solution, which occurs mainly by a singlet molecular oxygen [$O_2(^1\Delta_g)$] mechanism, that in homogeneous media, can be represented in terms of a simple Scheme:

$$O_2(^1\Delta_g) \xrightarrow{k_d} O_2(^3\Sigma^-{}_g) \quad (1)$$

$$P + O_2(^1\Delta_g) \xrightarrow{k_q} O_2(^3\Sigma^-{}_g) + P \quad (2)$$

$$P + O_2(^1\Delta_g) \xrightarrow{k_r} \text{Products} \quad (3)$$

where k_d is the solvent-dependent decay rate constant of singlet oxygen that determines its unperturbed lifetime ($\tau_o = 1/k_d$), k_q represents the second-order rate constant of physical deactivation and k_r is the second-order rate constant of the reactive pathway. The overall interaction between $O_2(^1\Delta_g)$ and P, comprises physical (Process 2) and chemical (reactive, Process 3) pathways, with a total reaction rate constant, $k_t = k_q + k_r$.

TN derivatives are essential indicators of thyroid gland diseases in clinical diagnosis and are currently used as standards

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¶Posted on the website on 11 January 2005

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Abbreviations: CT, charge transfer; D_2O, deuterium oxide; EO, eosin; ^{3}EO*, triplet excited state of eosin; FFA, furfuryl alcohol; ITyr, 3-iodotyrosine; I_2Tyr, 3,5-diiodotyrosine; NaN_3, sodium azide; $O_2(^1\Delta_g)$, singlet molecular oxygen; P, phenolic derivatives; PN, perinaphthenone; RB, rose bengal; ^{3}RB*, triplet excited state of rose bengal; SCE, saturated calomel electrode; TH, thyroidal hormones; TN, thyronine; TRPD, time-resolved phosphorescence detection; Tyr, tyrosine; T2, 3,5-diiodothyronine; T3, 3,3′,5-triiodothyronine; T4, 3,3′,5,5′-tetraiodothyronine or thyroxine; Xdye, xanthene dyes; ^{1}Xdye*, singlet excited state of xanthene dyes; ^{3}Xdye*, triplet excited states of xanthene dyes.

Table 1. Rate constants for reactive (k_r (M^{-1} s^{-1})) and overall (k_t (M^{-1} s^{-1})) quenching of $O_2(^1\Delta_g)$, thyronine hormones and related compounds; k_r/k_t values; rate constants for the quenching of $^3RB^*$ or $^3EO^*$ (k_{qT} (M^{-1} s^{-1})) and half-wave potentials for selected TH. The solvent was H_2O (k_r; k_{qT}; $E_{1/2}$ determinations) or D_2O (k_t determination) at pH or pD 12, unless other specified

Compound	k_r (a) or k_r α (b) × 10^{-7} ± 5%	k_t × 10^{-7} ± 10%	k_r/k_t (a) or k_r α/k_t (b) ± 10%	k_{qT} × 10^{-8}	$E_{1/2}$ (V) (±0.005)
Tyr	17*(a)	23	0.74 (a)		
ITyr	6.4 (a)	22	0.29 (a)		
I_2Tyr	4.3 (a)	21	0.21 (a)		
Ph*	15.5 (a)†	26.6‡	0.58 (a)		
TN	11 (b)	35	0.31 (b)	4.0 (EO)	0.293
T2	5.1 (b)	42	0.12 (b)		0.314
T3	4.7 (b)	50	0.09 (b)		
T4	3.8 (b)	62	0.06 (b)	22 (RB) 6.0 (EO)	0.323
PP	8.8 (b)	28	0.31 (b)		

*Ref. (17).

†Equivalent to pH 12, Ref. (6).

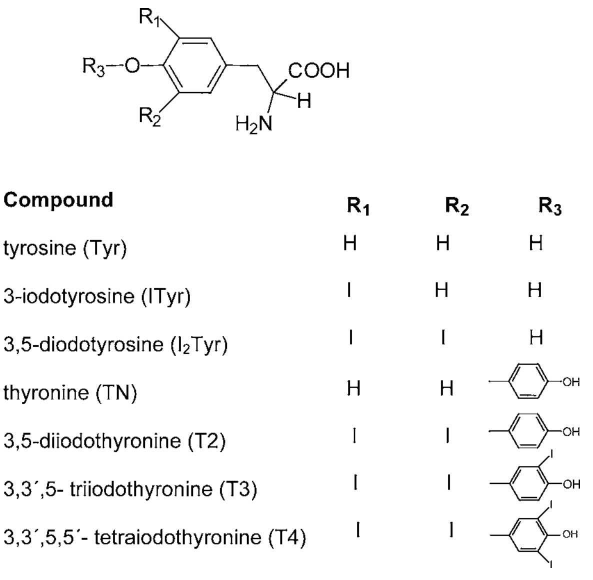
‡At pH 10.3, Ref. (31).

for developing ordinary biochemical assays (5). Among the physicochemical properties exhibited by these compounds is their ability to interact with *in vivo* oxidation–reduction systems, a topic that is still being discussed from long time ago (9–11). Relatively recent works relate the levels of the TH with the susceptibility of low-density lipoprotein to oxidation (12), long-chain fatty acid oxidation (11) and also with the oxidative damage in the organism, induced by free oxygen radicals (13). Hence, because of the biological relevance of these compounds and in the context of photosensitized oxidations, one objective of this study was the evaluation, in kinetic terms, of the potential activity of TH as antioxidants and their stability against $O_2(^1\Delta_g)$-mediated transformations on photoirradiation with environmental visible light in the presence of dye sensitizers able to absorb such an irradiation. This knowledge can contribute to the substantial understanding of unexpected biochemical transformations, which could take place as decomposition pathways of the hormones, due to photochemical instability either *in vivo* or under current laboratory conditions. Endogenous chromophores such as flavins and heme are good candidates to act as natural photosensitizers (14).

The phenolic amino acid tyrosine (Tyr) and some iodinated derivatives (also represented as TH, unless otherwise specified), all structurally related to TN hormones and whose photooxidative behavior is profusely described in the literature (8,15–17), have been included in this study for comparative purposes.

MATERIALS AND METHODS

Chemicals. Tyr, 3-iodotyrosine (ITyr), 3,5-diiodotyrosine (I_2Tyr), TN, T2, T3, T4, phenoxyphenol (PP), rose bengal (RB), eosin (EO), deuterium oxide (D_2O) (99.9% D) and fluorescamine were purchased from Sigma Chemical Co. (St. Louis, MO). Perinaphthenone (PN) and sodium azide (NaN_3) were from Aldrich (Milwaukee, WI). Furfuryl alcohol (FFA) was from Riedel de Häen (Seelze, Germany). Water was triple distilled. D_2O was used instead H_2O with the only objective to enlarge the lifetimes of $O_2(^1\Delta_g)$, especially in the time-resolved measurements (8). MeCN and MeOH (both of HPLC quality) were from Sintorgan (Buenos Aires, Argentina). Because of the low solubility of most TH derivatives in water, especially those that were iodine substituted, all experiments were carried out at pH or pD 12 by addition of the necessary amounts of KOH to the respective solvents. These solutions will be named ''alkaline solutions.''



Compound	R1	R2	R3
tyrosine (Tyr)	H	H	H
3-iodotyrosine (ITyr)	I	H	H
3,5-diodotyrosine (I2Tyr)	I	I	H
thyronine (TN)	H	H	OH
3,5-diiodothyronine (T2)	I	I	OH
3,3′,5- triiodothyronine (T3)	I	I	I OH
3,3′,5,5′- tetraiodothyronine (T4)	I	I	I OH I

Scheme 1. Chemical structures of thyronine and tyrosine derivatives.

Determination of overall quenching rate constants, k_t. The overall rate constant (k_t) was determined by time-resolved phosphorescence detection (TRPD), using a laser kinetic spectrophotometer. A nanosecond Nd:YAG laser system (Spectron) at either 532 or 355 nm was the excitation source. The emitted luminescence (mainly 1270 nm) was detected at right angles with an amplified Judson J16/8Sp germanium detector after passing through appropriate filters. The output of the detector was coupled to a digital oscilloscope and to a computer that allowed signal processing. Sixteen shots were usually needed for averaging decay times to get a good signal:noise ratio. The apparatus has been described previously (17). PN ($\Phi_\Delta = 1$ in D_2O [18]) with absorbances of about 0.4 at the laser emission wavelength was used as sensitizer. The k_t values were obtained from simple Stern–Volmer plots according to Eq. 4:

$$\tau^0/\tau = 1 + k_t\tau_0[\mathrm{TH}], \tag{4}$$

where τ and τ_0 are the $O_2(^1\Delta_g)$ lifetimes measured in the presence and absence of the quencher TH, respectively.

Steady-state photolysis and determination of photooxidation rate constants, k_r. The irradiation device for kinetic determinations, including the specific oxygen electrode (which can be used only in aqueous solutions), has been described elsewhere (17). A cut-off filter for 350 nm light was used.

The chemical rate constants k_r (using PN, $A_{400\ nm} = 0.5$, as a sensitizer) were measured using the method described by Foote and Ching according to Eq. 5 (19).

$$\mathrm{slope_{TH}}/\mathrm{slope_R} = k_r/k_{rR}, \tag{5}$$

which compares the slopes of the first-order plots (under pseudo first-order conditions) for the substrate and the reference oxygen uptake kinetics ($\mathrm{slope_{HT}}$ and $\mathrm{slope_R}$, respectively). The reference used was FFA ($k_{rR} = 1.2 \times 10^8$ M^{-1} s^{-1}, Ref. 20).

In our case, because of experimental reasons, the consumption of oxygen was monitored instead of substrate consumption. Assuming that the reaction of $O_2(^1\Delta_g)$ with the quencher is the only channel for oxygen consumption, the ratio of the first-order slopes of oxygen uptake by reference and substrate, both at identical concentrations ($\mathrm{slope_{TH}}/\mathrm{slope_R}$), yields $\alpha k_r/\beta k_{rR}$, where α and β represent the respective stoichiometric coefficients in the reactions:

$$\mathrm{TH} + \alpha O_2 \rightarrow \mathrm{Products\ TH} \tag{6}$$

$$\mathrm{R} + \beta O_2 \rightarrow \mathrm{Products\ R} \tag{7}$$

To use this experimental technique, the values of α = (mol O_2/mol TH) and β = (mol O_2/mol R) on sensitized irradiation are required. For FFA (the

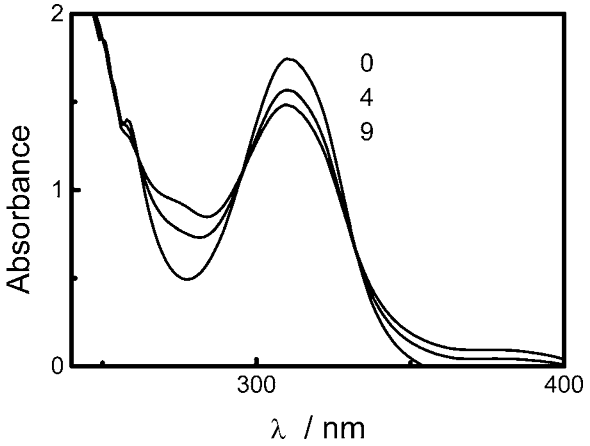


Figure 1. Spectral evolution of I_2Tyr sensitized by perinaphthenone, on visible light irradiation, in water–KOH 10 m*M*. Numbers on the spectra represent irradiation time (minutes).

reference), the stoichiometric quotient is unity (20). This quotient was determined for each substrate investigated by measuring the respective molar consumption of TH and oxygen at the initial stages in the same photoirradiated solution.

Absorption measurements were carried out with a Hewlett Packard 8453A diode array spectrophotometer.

Experiments on primary amine reactivity. The rates of evolution of primary amine reactivity in the substrates (initial concentrations 2×10^{-4} *M*) on RB-sensitized photooxidation were determined using the method described by Straight and Spikes (21). Basically, the method uses the specific complexing agent, fluorescamine, which produces a fluorescent complex in the presence of primary amino groups. In this manner, it is possible fluorimetrically to monitor the evolution of primary amino groups (either $-NH_2$ generation or consumption) on sensitized photoirradiation of the substrate. The spectrofluorometer used was a Spex Fluoromax, and the excitation and emission wavelengths for the complex [NH_2-fluorescamine] were 390 and 475 nm, respectively.

Determination of rate constants for quenching of triplet excited state of RB and EO. Nitrogen-saturated RB or EO (xanthine dyes [Xdye]) aqueous solutions, of absorbance *ca.* 0.2 at the lasing wavelength, were photolyzed using a laser flash photolysis apparatus. A nanosecond Nd:YAG laser system (Spectron) at 532 nm was the excitation source, using a 150 W xenon lamp as the analyzing light. The detection system comprised a PTI monochromator and a red-extended photomultiplier (Hamamatsu R666 Shimokanzo, Japan). The signal, acquired and averaged by a digital oscilloscope (Hewlett–Packard 54504A), was transferred to a personal computer via a HPIB parallel interface, where it was analyzed and stored.

The aqueous solutions were deoxygenated by bubbling with ultrapure solvent-saturated argon for 30 min. Transient absorbance decays were fitted to a monoexponential decay plus a constant term.

The triplet excited state of RB (^{3}RB*) or EO (^{3}EO*) (generically called ^{3}Xdye*) was generated by laser excitation, and the spectrum of the transient species was coincident with the literature reports (20,22,23). The rate constants for the quenching of ^{3}RB* (k_{qT}) were obtained through a simple Stern–Volmer treatment (Eq. 8) by determining the triplet lifetimes of RB in the absence ($\tau_T{}^o$) and in the presence (τ_T) of different concentrations of TH in argon-saturated solutions.

$$\tau_T^0/\tau_T = 1 + k_{qT}\tau_T^0[\mathrm{TH}] \qquad (8)$$

Determination of the half-wave potentials. Electrochemical measurements were performed in a two-compartment Pyrex cell. The working electrode was a Pt wire. Before each measurement, the Pt wire electrode was cleaned by heating to the red heat in a burner flame. The counter electrode was a platinum foil of large area (approximately 2 cm^2). The reference electrode was an aqueous saturated calomel electrode (SCE). Solutions were deaerated by bubbling pure nitrogen. All measurements were performed at 25 ± 0.5°C in H_2O–KOH 10 m*M* as a solvent.

The convolution of Faradaic current was calculated by applying the algorithm proposed by Oldham to the background-corrected voltammograms (24). The measuring system for linear scan voltammetry (LSV) was constructed from an EG&G PARC Model 273 potentiostat run with model PAR270 electrochemical analysis software.

RESULTS

PN-photosensitized process

The visible light irradiation of air-equilibrated individual solutions of TH in aqueous alkali solutions, in the presence of PN under sensitizing conditions, results in a change in the absorption spectrum of the solutions (Fig. 1). These spectral modifications were totally absent when argon-saturated solutions were photoirradiated.

In a separate set of experiments, the rate of oxygen consumption on irradiation of alkaline-aerated PN–TH systems was recorded using the specific oxygen electrode. Oxygen uptake was totally inhibited by 0.01 *M* NaN_3, a known selective $O_2(^1\Delta_g)$ physical quencher (8). All the experimental facts agree with the currently accepted criteria for the occurrence of a Type-II ($O_2(^1\Delta_g)$ mediated) photooxidation, unambiguously corroborated later by the time-resolved $O_2(^1\Delta_g)$–luminescence quenching experiments (16).

RB and EO photosensitization

Using the xanthene derivatives RB or EO (Xdye), two recognized $O_2(^1\Delta_g)$ dye sensitizers (25), we clearly observed spectral modifications in both aerated and argon-bubbled solutions of TH, with the changes in the deoxygenated runs some different in shape and magnitude to those obtained in the presence of oxygen. This fact shows that the Xdye-sensitized process of TH cannot be interpreted through an exclusive $O_2(^1\Delta_g)$-mediated pathway and that other interactions between excited singlet (^{1}Xdye*) or excited triplet (^{3}Xdye*), or both, could also be operative. Nevertheless, the possibility of a ^{1}Xdye* quenching (Process 9) should be disregarded in the range of TH concentrations used in this study: the reported lifetime of ^{1}RB* and ^{1}EO* in aqueous solutions is 0.82 ns and 3.6 ns, respectively (26).

$$^1\mathrm{Xdye}^* + \mathrm{TH} \xrightarrow{k_{qS}} \mathrm{Products} \qquad (9)$$

This means that the singlet excited species of the sensitizers, even assuming diffusional k_{qS} values (Eq. 9), cannot be intercepted by TH at concentrations in the order of 0.1 m*M* as those used in the determination of spectral changes on irradiation.

The presence of TH, in the order of 1 m*M* or higher, in Xdye aqueous solutions appreciably decreases the triplet lifetime, supporting the interaction depicted by Eq. 10. The respective k_{qT} values for some representative TH obtained through laser flash photolysis experiments (Fig. 2, inset) are shown in Table 1.

$$^3\mathrm{Xdye}^* + \mathrm{TH} \xrightarrow{k_{qT}} \mathrm{Products} \qquad (10)$$

The oxygen uptake during RB-sensitized photoirradiation of TH was partially inhibited by NaN_3. A maximum effect of only *ca.* 80% was reached at 5 m*M* NaN_3. This fact is indicative that not all consumed molecular oxygen is the result of a $O_2(^1\Delta_g)$-mediated pathway and that some additional source of oxygen uptake must be present.

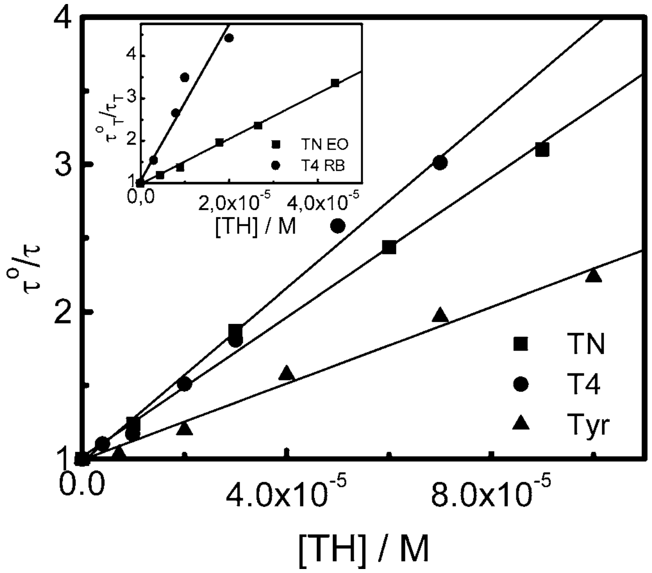


Figure 2. Stern–Volmer plots for the quenching of $O_2(^1\Delta_g)$ luminescence by TN, T4 and Tyr. Inset: Stern–Volmer plots for the quenching of $^3RB^*$ by T4 and $^3EO^*$ by TN (Argon-saturated solutions).

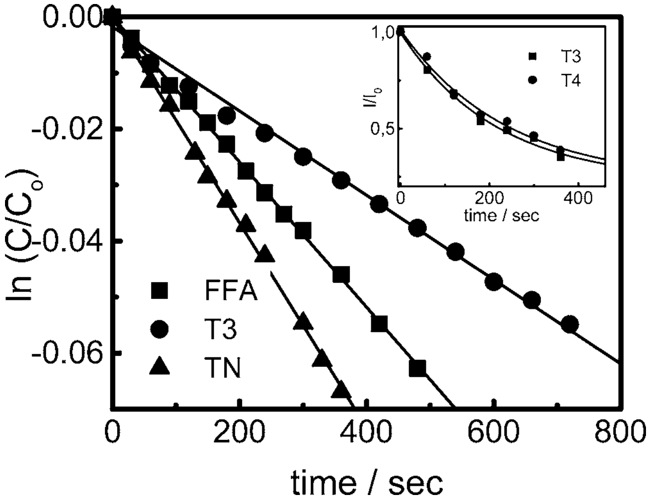


Figure 3. First-order plots for oxygen uptake by T3, T4 and FFA on perinaphthenone-sensitized photoirradiation in water–KOH 10 m*M*. Inset: evolution of primary amino group in T3 and T4, on RB-sensitized photoirradiation in water–KOH 10 m*M*. I and I_0 represent fluorescence intensities at irradiation time t = t and t = 0, respectively (see Materials and Methods).

Determination of k_t and k_r values

In view of the preceding results, we decided to use only PN as a dye sensitizer for the evaluation of rate constants for the $O_2(^1\Delta_g)$-mediated photooxidation of TH.

The data obtained for TH, through the TRPD method, were treated according to Eq. 4 (see Fig. 2 for typical examples). Table 1 shows the k_t values.

The k_r values for TH (Table 1) were obtained through experiments of oxygen uptake in alkaline solutions. Typical examples are shown in Fig. 3. The stoichiometric factors (α, Eq. 6) were evaluated by simultaneous quantification of moles of oxygen consumed and moles of substrate consumed at a given irradiation time. Although substrate consumption on photoirradiation for Tyr derivatives could be evaluated from their respective absorption spectra (Fig. 1), the presence of UV-absorbing reaction products in the TN family, in particular at high conversions, prevents the neat evaluation of substrate disappearance from UV data. The stoichiometric quotient (α) for the reaction between $O_2(^1\Delta_g)$ and Tyr derivatives was 1.5 ± 0.1 (data not shown). This value is coincident with data for other Tyr derivatives (17). For the TN family the coefficients α were not evaluated, and the product $k_r\alpha$ is reported in Table 1.

As was stressed elsewhere, no relevant information about the effectiveness of the actual photodegradation is obtained either from the overall k_t or from the reactive rate constant k_r in a $O_2(^1\Delta_g)$-mediated photooxidation (7). This information is only given by the photooxidation quantum efficiencies (φ_r) (Eq. 11), which takes into account the simultaneous effect of the physical and chemical interaction. The first contribution constitutes, in practical terms, a form of self-protection against mediated photooxidation.

$$\varphi_r = k_r[\mathrm{TH}]/(k_d + k_t[\mathrm{TH}]), \qquad (11)$$

where k_d is the rate constant for $O_2(^1\Delta_g)$ deactivation because of interaction with solvent molecules.

Expression (12) is somewhat limited because of its dependence on the concentration of the photooxidizable substrate ([TH]), which is unknown in many cases, especially in complex biological environments. A more simple and useful approach is the k_r/k_t ratio, which is the fraction of overall quenching that effectively leads to chemical reaction.

The k_r/k_t ratio for TH in alkaline solution was included in Table 1.

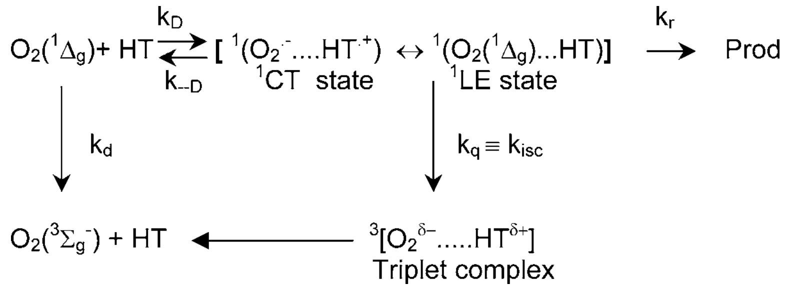
Evolution of primary amino groups

The evolution of the reactive NH_2 groups during the sensitized photooxidation of T3 and T4, under identical experimental conditions (in pH 12 water), determined by fluorimetric analysis (see Materials and Methods), is shown in Fig. 3, inset. The trend is qualitatively coincident with the kinetic profile of Tyr, which is already published (17). The observed behavior for the hormones was reproducible within 3%. There is a continuous decrease in the fluorescence signal as the photooxidation progresses, indicating the simultaneous disappearance of primary amino groups. This technique has been previously used with success by the authors to demonstrate the evolution of peptide bonds on sensitized photooxidation of amino acids and peptides (17,27).

Determination of half-wave potentials

Because of the possible involvement of charge transfer (CT)–type components in the $O_2(^1\Delta_g)$–TH interactions, the oxidation potentials of some selected TH derivatives were determined.

Linear scan voltammograms for the electrooxidation of TN, T2 and T4 were obtained at a scan rate (v) of 0.05 V s^{-1}. A well-defined anodic oxidation peak in the potential region around 0.30 V *vs* SCE was observed. Because the peak potentials of TN, T2 and T4 are similar, it is difficult to compare them from the linear scan voltammograms. So, linear voltammograms, after background current subtraction, were convoluted. Advantages that the con-



Scheme 2. Possible mechanism for the quenching of singlet molecular oxygen [$O_2(^1\Delta_g)$] by TH.

volution technique offers in the treatment of experimental data from linear sweep voltammograms are well known (28).

The convoluted current, I(t), for TN, T2 and T4, reaches a limiting or maximum value, I_L, which is characteristic of a purely diffusion-controlled process. If the reaction is Nernstian, the dependence of the potential, E, with I(t) is expressed by Eq. 12 (28):

$$E = E_{1/2} - (RT/nF)\ln(I_L - I(t))/I(t) \qquad (12)$$

where $E_{1/2}$ is the half-wave potential, n is the number of electrons exchanged per molecule of compound oxidized and the other terms have their usual meaning.

A plot of E *vs* $I_L - I(t)$ is a straight line. Thus, $E_{1/2}$ could be obtained from the intercept of that plot. Linear plots were obtained from postconvolution analysis of E *vs* ln $(I_L - I(t))$ in the 90% of the curve for TN, T2 and T4. The values of $E_{1/2}$ from experimental plots with regression parameters better than 0.9992 are in Table 1.

DISCUSSION

Photodynamic action, describing damaging effects in compounds of biological relevance by visible light irradiation in the presence of RB or EO as dye sensitizers, has been extensively reported (for a review see Ref. 8). Nevertheless, the photooxidation mechanism in amino acids, peptides, proteins and vitamins, among other biomolecules studied, does not always correspond to pure Type-II ($O_2(^1\Delta_g)$ mediated) or Type-I (radical mediated) events (16). This is the case of Xdye-sensitized photooxidation of TH. An important contribution of a second source of oxygen consumption, different from the $O_2(^1\Delta_g)$ process, involving reactions between ^{3}Xdye* with ground-state substrates is present in the case of the TH compounds. This proposition is supported by at least two facts. The first one that accounts for the lack of suppression of oxygen uptake by the $O_2(^1\Delta_g)$ quencher, NaN_3, on RB-sensitized irradiation of TH, is not a novel result: it is known that the quenching of the ^{3}RB* by ground-state oxygen leads to approximately 75% $O_2(^1\Delta_g)$ and 25% $O_2^{\bullet-}$. This finding comes from Lee and Rodgers although it was initially reported in a qualitative fashion by Srinivasan *et al.* (29,30). The second fact, represented by the reactive interaction of ^{3}Xdye* with TH (Eq. 10), could be attributed to an electron transfer process, with the TH as electron donors, in a mechanism similar to that reported by Zwicker and Grossweiner, Okamoto *et al.* and Lambert and Kochevar for interactions of ^{3}EO* and ^{3}RB* with P (23,31,32). Rate constants for Process 10 are very close to those included in Table 1. Furthermore, a value of ΔG *ca* –0.3 eV, demonstrating the feasibility of the electron transfer process, has been reported by Lambert and Kochevar for the reductive quenching of ^{3}RB* by Tyr (32).

Using the carbonyl dye PN as a sensitizer, no evidences for additional reactions different from those corresponding to a pure Type-II mechanism were found. From the analysis of the kinetic data collected in Table 1, several remarkable points arise. (1) The overall rate constant k_t increases with the number of iodine substituents in the TH skeleton in the TN series. This behavior parallels the increase of the oxidation potential in the TN derivatives studied. (2) The k_r values decrease with the number of iodine substituents in the Tyr series. If a similar α value for all the TN compounds is assumed, the same trend could be observed in the TN series. (3) The quotient k_r/k_t, accounting for the efficiency of the photooxidative process in TH, decreases with the number of iodine substituents. The higher values for the quotient correspond to the noniodinated compounds. The k_r/k_t ratios are also very close or coincident for the pairs Tyr–P and TN–PP. In both couples, these noniodinated compounds differ structurally by the presence or not of the amino acid chain in the *p*-position. In other words, the physical quenching component is mainly due to interactions of $O_2(^1\Delta_g)$ with the aromatic moiety.

To rationalize the above results the following considerations should be taken into account.

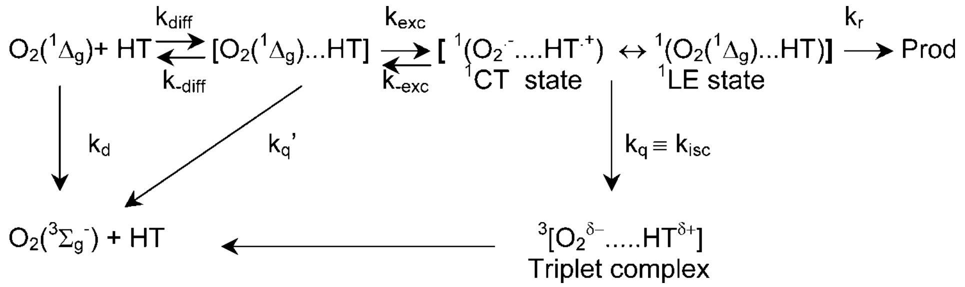
It has been established [7] that the process of $O_2(^1\Delta_g)$ quenching by phenols (Q) is because of the presence of the OH group in the aromatic ring. It occurs through an encounter complex $[O_2(^1\Delta_g)\ldots Q]$ (33–35) (Scheme 2).

The physical quenching results from the spin-orbit coupling–induced intersystem crossing within the complex, which must possess some degree of CT (35). Hence, the balance between physical quenching and effective oxidation (products generation) should be sensitive to the spin–orbit coupling and entropy factors. All these conclusions were raised mainly by Gorman *et al.* (33,34), and some are supported by solvent (36) and temperature effects on rate parameters of selected model compounds.

Scheme 2 is based on the original approximation of Rehm and Weller (37), where k_{ISC} is the instersystem-crossing rate constant and CT state and LE state refer to charge-transfer and locally excited states.

According to this mechanism, both chemical reaction and physical quenching can operate; the relative importance of each process is reflected by the relative magnitudes of the rate constants k_r and k_q.

If intersystem crossing depends on the CT process, k_{ISC} should increase when the CT character of the exciplex increases as a result of the increase in the spin–orbit coupling component. In parallel, the CT degree should increase as the oxidation potential of the donor compound (Q) decreases.



Scheme 3. Modified Scheme 2.

The increase in reactivity toward $O_2(^1\Delta_g)$ by the ionized species as compared with the neutral ones is a very well known fact for a great number of phenols (7). Considering that TN are substituted phenols, Scheme 2 is applicable to the $O_2(^1\Delta_g)$ quenching process for these compounds. Nevertheless, for the group of TH for which the $E_{1/2}$ has been determined, it is evident that k_t increases with the increase of $E_{1/2}$. This fact suggests that another process, besides the CT process, dominates the $O_2(^1\Delta_g)$ quenching and demands a further analysis of the situation.

The physical deactivation of $O_2(^1\Delta_g)$ is also a singlet–triplet intersystem crossing (S–T), followed by vibrational relaxation of the ensuing excited triplet state O_2 molecule. The rate constant for the process (k_{ISC}) is known to be controlled by two factors. One is the electronic coupling constant, which gives the preexponential factor, and the other is the nuclear reorganization related to the energy barrier for the process (38). Because the S–T process is spin forbidden, the magnitude of the preexponential factor is expected to be dependent on the heavy atom effect. It should be given by the number and position of the halogen substituents in the TH.

Thus, k_{ISC} can be written as

$$k_{ISC} = B\Sigma(\xi)^2 \exp(-\Delta E^*/RT), \qquad (13)$$

where B is a constant and ξ are the respective spin–orbit constants of the halogen substituents. The summation is over the number of halogen atoms in the TH.

Thus, Eq. 13 indicates that an increase in the $\sum \xi^2$ will produce a concomitant increase in the k_q value.

On the other hand, and turning again to Scheme 2, it appears that there should be an additional contribution to intersystem crossing from the encounter complex preceding formation of the exciplex, produced by the heavy atom effect, and increasing the value of the $O_2(^1\Delta_g)$ physical quenching. Obviously, this factor does not operate in the absence of the heavy atoms.

This proposal introduces some modifications to Scheme 2 as shown in Scheme 3.

Scheme 3, in which k_{diff} is the diffusion-controlled rate constant and k_{exc} is the rate constant for the exciplex formation, suggests two possible pathways for physical deactivation of $O_2(^1\Delta_g)$, represented by k_q and k_q', which are competitive with product formation k_r. Consequently, as the number of heavy atoms increases the rate constants for total $O_2(^1\Delta_g)$ quenching increase, despite the increase in the respective $E_{1/2}$ values. Furthermore, the k_r values decrease because of two factors: the increase in $E_{1/2}$ and the progressive depopulation of the encounter complex because of a competitive pathway for products formation. This behavior has already been predicted (34,39) but never confirmed with experimental data, according to our knowledge. The noniodinated compounds that differ only in the presence of the amino acid residue obviously do not suffer any heavy atom assistance and behave kinetically in a similar fashion regarding the k_r/k_q ratio.

This residue, present in almost all the TH studied, participates in the generation of the oxidation products, as suggested by the fluorescamine experiments. Besides, the relative rates of primary amino group consumption, estimated from the initial slopes of the corresponding consumption curves (inset Fig. 3), parallel the respective k_r values, as shown for T3 and T4 in Fig. 3.

We did not attempt the elucidation of TH photooxidation products. Product analysis in the photooxidation of phenolic compounds by $O_2(^1\Delta_g)$ has been carried out for different members of the family, such as the parent compound phenol (31), alkyl-substituted phenols (36) and dihydroxybenzenes (40). In all cases, the main product detected possesses a *p*-quinonic structure. Nevertheless, Gerdes *et al.* postulate that in alkaline media, which is the case of our experimental conditions, the *p*-benzoquinone reacts with the species $O_2(^1\Delta_g)$ and OH^- to give highly oxidized products of low molecular weight (41). The generation of a mixture of polymeric products on prolonged sensitized irradiation was reported for phenol in neutral medium (31). The phenolic amino acid Tyr exhibits a different reaction pattern, which, according to the mechanism proposed by Katsuya *et al.* (42), includes the formation of a lactame as a final product. In the case of the sensitized photooxidation of Tyr peptides, in alkaline medium, the primary products proceed with the breakage of peptide bonds, being the products distribution highly dependent on the extent of the photoconversion (15). As can be seen, a common characteristic for all phenolic compounds, which may be also the case for TH, is that primary photoproducts are further photooxidized to give a complex mixture of final and intermediate products. This situation turns very difficult for the evaluation or confirmation of a given reaction mechanism in accordance with kinetic evidence, on the basis of the structure of generated products.

CONCLUSIONS

The photooxidation of TH, sensitized by the Xdye EO or RB, starts a complex combination of competitive reactions involving Type-I and Type-II photoprocesses.

The process seems to be a pure Type-II–mediated mechanism using the carbonyl sensitizer PN. The presence of the phenolic group in Tyr, TN and iodinated derivatives dominates the kinetics of the photooxidative events. Reactive interaction is only reached at sufficiently high pH values in which the phenol moiety is ionized. Besides, the respective rate constants for the overall interaction, k_t, increase with the oxidation potential of the TH and subsequently with the number of iodine substituents in the aromatic rings. Nevertheless, the reactive rate constants k_r behave in a different fashion compared with the overall ones. This apparent inconsistency can be interpreted on the basis of an internal heavy atom effect, favoring the intersystem-crossing deactivation route within the encounter complex and concomitantly reducing the effective photooxidation.

Acknowledgements—We thank Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT) and Secretaría de Ciencia y Técnica de la Universidad Nacional de Río Cuarto (SECyT UNRC), all from Argentina for financial support. S. M. acknowledges FOMEC-UNRC for financial travel assistance.

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