Amino Acids (2008) 35: 201–208
DOI 10.1007/s00726-007-0566-4
Printed in The Netherlands

Amino Acids

Singlet molecular oxygen [$O_2(^1\Delta_g)$]-mediated photodegradation of tyrosine derivatives in the presence of cationic and neutral micellar systems

S. Criado[1], **J. P. Escalada**[2], **A. Pajares**[2], and **N. A. García**[1]

[1] Departamento de Química, Universidad Nacional de Río Cuarto, Río Cuarto, Argentina
[2] Unidad Académica Río Gallegos, Universidad Nacional de la Patagonia Austral, Río Gallegos, Argentina

Received March 20, 2007
Accepted May 14, 2007
Published online July 6, 2007;

Summary. The kinetics of rose bengal-sensitized photooxidation of tyrosine and several tyrosine-derivatives (tyr-D) named tyrosine methyl ester, tyrosine ethyl ester and tyrosine benzyl ester was studied in buffered pH 11 water, and buffered pH 11 micellar aqueous solutions of 0.01 M cetyltrimethylammonium chloride (CTAC) and 0.01 M-octylphenoxypolyethoxyethanol [triton X100 (TX100)]. Through time-resolved phosphorescence detection of singlet molecular oxygen ($O_2(^1\Delta_g)$) and polarographic determination of oxygen consumption, the respective bimolecular rate constants for reactive (k_r) and overall (k_t) quenching of the oxidative species by tyr-D were evaluated. Both rate constants behave in different fashion depending on the particular reaction medium. k_r/k_t values, increase in the sense CTAC$\ll$TX100 $<$ water, indicating, for the tyr-D family studied, an excellent degree of *self-protection* against $O_2(^1\Delta_g)$-attack in the CTAC micellar system and a high photooxidability level in water. Results were interpreted in terms of a competition between solvent polarity effects, local substrate concentration and electron donating capabilities of the substrates in the different media that can contribute to predict the extent of photodynamic damage in biological environments.

Keywords: CTAC – Micelles – Photooxidation – Singlet molecular oxygen – Tyrosine – Tyrosine-derivatives – Triton X100

Abbreviations: CTAC, cetyltrimethylammonium chloride; $O_2(^1\Delta_g)$, singlet molecular oxygen; $O_2(^3\Sigma_g^-)$, ground state triplet oxygen; TX100, t-octylphenoxypolyethoxyethanol; tyr, tyrosine; tyrBzE, tyrosine benzyl ester; tyr-D, tyrosine derivatives; tyrEE, tyrosine ethyl ester; tyrME, tyrosine methyl ester

Introduction

Model studies of the sensitized-visible light mediated-photodamage in proteins mainly consider the chemistry of the oxidation of free amino acids (AAs). Tyrosine (tyr) residues represent an important site of the so-called photodynamic effect in proteins, since it is one of the few AAs susceptible to singlet molecular oxygen [$O_2(^1\Delta_g)$]-mediated photooxidation, Straight and Spikes (1985), Parker et al. (2004), Svistinenko (2005) and Montfort et al. (2006). For this reason, a number of works have been devoted to the elucidation of the reaction mechanism, reaction products and kinetic aspects of the photodegradative process in isolated tyr, tyr-derivatives (tyr-D) and tyr-containing small peptides, Bertolotti et al. (1991), Miskoski et al. (1993), Michaeli and Feitelson (1994), Criado et al. (1995), Soltermann et al. (1995) and Criado et al. (1998, 2001). It is a crucial objective, in this area, to establish possible relationships between substrate structure and susceptibility to photodynamic action. One possible approach to this study, in complex biological assemblies is the investigation of kinetics and mechanism involved in the photooxidation of a given model system. Published results indicate that the characteristics of the reaction medium operate on the kinetics of the photodynamic event, such as solvent polarity and pH effects. Since proteins, and particularly tyr residues, can occupy different locations in complex biological systems, kinetic information about the visible-light promoted interactions between these compounds under given environmental conditions can help to know more on the chemical and physical behaviors of activated oxidative species in general, on the potential photoreactions of said tyr-residues in particular and on the propensity of all these process to occur as a function of the external conditions. The dramatic increase of tyr photooxidability with the ionization of its phenolic OH group is known (García, 1994). On this basis we report, in the present paper, a comparative kinetic study, in homogeneous and microheterogeneous media at pH 11, that essentially includes the evaluation of the photooxida-

tion efficiencies under pH conditions that maximize the reactivity of the family of tyr-D, formed by tyr, tyrosine methyl ester (tyrME), tyrosine ethyl ester (tyrEE) and tyrosine benzyl ester (tyr-BzE), towards $[O_2(^1\Delta_g)]$, employing water, the positively charged cetyltrimethylammonium chloride (CTAC) and the neutral t-octylphenoxypolyethoxyethanol, Triton X-100 (TX100) detergents as reaction media.

Micelles have been proposed as a primitive model for the environment represented by biological systems and constitute an attractive mimicking for biomembranes, Chattopadhyay et al. (2002). They possesses a number of important and essential features of these non simple systems, although lacking much of the complexity associated with the biological assemblies. Reactions in detergents may strongly reflect constraints imposed by their micellar environment, such as local concentration and stabilization of ionic species. This information is particularly important since a substrate incorporated into a given microheterogeneous structure may be located in a variety of media that could deeply affect its reactivity. In this context, we think that the information about kinetic aspects of the photodynamic effect in combination with the interaction of organized media with amino acids would provide valuable information for deeper understanding on the effects of photosensitized reactions in biological environments and constitute an intermediate step in the extrapolation to more complex systems. Although the pH conditions used in this study may not be considered suitable for a study in biological systems, in this particular case, it allowed an exacerbation of tyr photodynamic susceptibility, simplifying the kinetic study of neat oxidative reactions.

Materials and methods

Materials

Tyrosine, tyrosine methyl ester, tyrosine ethyl ester, tyrosine butyl ester, tyrosine benzyl ester, triton X100, sodium azide (NaN_3) and deuterium oxide 99.9% (D_2O), were purchased from Sigma Chem. Co., Rose Bengal (RB) was from Aldrich and furfuryl alcohol (FFA) was from Riedel de Haën. All these chemicals were used as received N-phenyl-1-naphthylamine was purchased from Merck. It was recrystallized from methanol and subsequently vacuum sublimed. Purified CTAC was a gift from Dr. M. Hamity. Water was triply distilled. All the measurements were carried out at room temperature and with freshly prepared solutions. Buffered aqueous solutions (pH 11) were prepared with Na_2HPO_4 0.05 and NaOH 0. 1 M, Weast (1963).

Methods

Absorption spectra were registered with a Hewlett Packard 8452A diode-array spectrophotometer. Continuous photolysis was performed in a home-made photolyser with a 300-W quartz-halogen lamp and a cut-off filter at 360 nm, using RB ($Abs_{530} = 0.5$) as a sensitizer. The overall quenching rate constant k_t of the $O_2(^1\Delta_g)$-deactivation by each tyr-D (see Scheme 1) was determined as reported elsewhere, Neumann and García (1992). Briefly, RB was excited with the frequency doubled output at 532 nm, of a Nd:YAG laser (Spectron). The emitted $O_2(^1\Delta_g)$-phosphorescence at 1270 nm was analyzed by time-resolved phosphorescence detection (TRPD) at right angle using a Judson J16/8Sp germanium detector, after passing through the appropriate filters. The amplified output of the detector was coupled to a digital oscilloscope and to a personal computer to process the decay signal. Usually, averaging 10 laser shots was enough to get a good signal-to-noise ratio. Air-saturated solutions were employed in all the cases. RB ($Abs_{530} = 0.3$) in air-equilibrated solutions were used as sensitizers. In the TRPD determinations, D_2O was employed as solvent in order to enlarge the $O_2(^1\Delta_g)$ lifetime, Nonell et al. (1995). $O_2(^1\Delta_g)$ decay lifetimes were evaluated in the presence (τ) and in the absence (τ_0) of quencher; the data were analyzed as a function of tyr-D concentration, according to a simple Stern-Volmer expression: $1/\tau = 1/\tau_0 + k_t[\text{tyr-D}]$. The value of k_r for the reaction of each tyr-D with RB-generated $O_2(^1\Delta_g)$ was determined by a relative method, using the expression: $\text{slope}/\text{slope}_R = k_r[\text{tyr-D}]/k_{rR}[R]$, and assuming that the reaction of $O_2(^1\Delta_g)$ with the quencher (reaction (4)) is the only pathway of oxygen consumption, Scully and Hoignè (1987). Briefly, in this method the slope of the first order plot of oxygen consumption by each tyr-D (slope) and by a reference compound R (slope_R) are determined experimentally in the same conditions of temperature and concentration. The reference compound used here was furfuryl alcohol (FFA), with determined rate constant values in the different media, as follows: $k_{rR\ water} = 2.4\times10^8\ M^{-1}\ sec^{-1}$, $k_{rR\ TX100} = 1.1\times10^8\ M^{-1}\ sec^{-1}$ and $k_{rR\ CTAC} = 1.2\times10^8\ M^{-1}\ sec^{-1}$ all prepared in pH (water)/pD (detergents) 11 buffered aqueous solutions in the presence of detergent concentration 10 mM.

Determination of CMC

In this study, the critical micellar concentration (CMC) in CTAC and in TX100, both in pH 11 buffered aqueous solution, in the presence of 0.5 mM tyr was determined with a well established method, from the concentration of detergent at which a sharp increase in the fluorescence intensity of N-phenyl-1-naphthylamine (0.01 mM) begins, Bertolotti et al. (1989a). The fluorescence quantum yield of this compound is highly dependent on the solvent polarity and therefore it can be used as a probe for CMC determination. Fluorescence intensity was measured with a Spex Fluoromax spectrofluorometer at $25 \pm 1\,^\circ C$ in air-equilibrated solutions with excitation and emission wavelengths of 350 and 408 nm, respectively.

Results

Interaction of anionic tyr-derivatives with CTAC micelles

It has been well established that the species involved in the sensitized photooxidation of phenolic compounds in general, and tyr-D in particular, are the phenolate ions, Bertolotti et al. (1991), García (1994) and Criado et al. (1998, 2001). In order to maximize the oxidative interaction, in principle attributed to a $O_2(^1\Delta_g)$-mediated reaction, we exclusively worked in buffered pH 11 aqueous solutions, García (1994) and Wilkinson et al. (1995). According to reported pK_a values for the phenolic OH group, tyr-D are mostly in their anionic form at this pH, Weast (1963) and Criado et al. (1995).

The UV spectra in pH 11 buffered aqueous solution of all tyr-D (ca. 0.5 mM) studied exhibited a red shift in a

10 mM CTAC micellar medium as compared to those in pH 11 aqueous solution, as shown in Fig. 1 inset I, for the case of tyr. The same was true for RB, the photosensitizer employed in this work, as can be observed in inset II of Fig. 1. CTAC is a cationic detergent, forming polar, positively headed micelles, with Cl^- as a counterion, whereas TX100 forms non-ionic micelles. Results clearly indicate an interaction between the tyr-D employed in this work and micellised CTAC, as previously reported for other phenols, Bertolotti et al. (1989b) and Luiz et al. (2002). No spectral shifts were observed, neither in tyr-D nor in RB, when CTAC was replaced by 10 mM of TX100, always at pH 11, with exception of tyrBzE for which a very small blue shift was observed.

The concentration of both detergents employed ensures that micelles are completely formed in pure water. Nevertheless, the increased solubility of some compounds in the presence of micelles may affect the micellar volume and could change the number of surfactants units associated with the micelle. In order to determine the actual CMC values in the presence of tyr-D, the experiments described in the methodology section were done (Fig. 2, inset), employing tyrME as a representative compound (see Fig. 2, inset). Results indicate that CMC of TX100 remains in its value for pure water (ca. 0.3 mM), whereas for CTAC decreases from 1.4 mM in pure water to 0.085 mM in the presence of the tyr-D (data not shown), Fendler (1982). This tendency is in agreement with published data: for tryptophan 0.4 mM in 10 mM TX100, pH 7, the reported CMC value was 0.34 mM and for chlorophenols 0.1 mM in CTAC, pH 10, the reported CMC value was 0.06 mM, Bertolotti et al. (1989b) and Criado et al. (1997).

Sensitized photoirradiation of tyr-D in water and in micellar solutions

Individual solutions of tyr-D either in water or in the micellar solutions, were irradiated, with visible light, in the presence of the well known $O_2(^1\Delta_g)$ sensitizer RB ($Abs_{534\,nm} = 0.5$). The dye is an efficient and almost exclusive $O_2(^1\Delta_g)$ photogenerator, with a quantum yield 0.7 in water, Amat-Guerri et al. (1990) and Wilkinson et al. (1995). Important changes were observed in the respective absorption spectra of the tyr-D (see main Figs. 1 and 2). As control experiments, solutions of RB ($Abs_{534\,nm} =$ 0.5–0.6), in pH 11 aqueous solution in the presence and in the absence of 0.01 M CTAC or 0.01 M TX100, and always in the absence of tyr-D, were photoirradiated. It is important to emphasize that in all three cases no modifications were observed in the whole absorption spectrum of the sensitiser, even employing ten-fold higher irradiation doses than those described in Figs. 1 and 2 for the photooxidation of tyr-D.

Although in homogeneous media the reactivity of the mentioned tyr-D towards $O_2(^1\Delta_g)$ is known, several classic complementary experiments were carried out in order to confirm this fact, both in the absence and in the presence of the surfactant, as follows: a) the reaction was totally suppressed in the absence of dissolved molecular

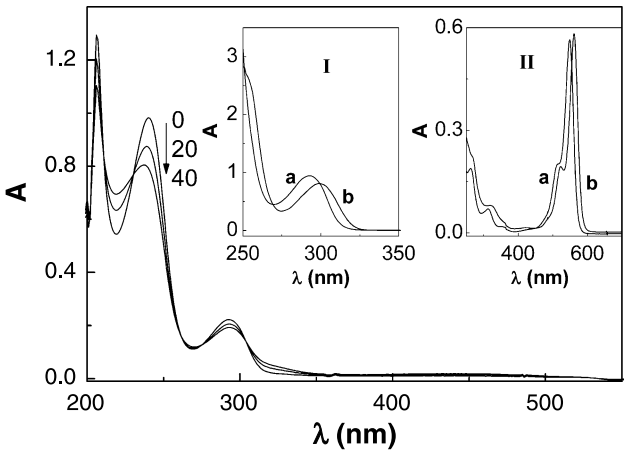

Fig. 1. Spectral changes of tyrosine 0.1 mM in air-equilibrated aqueous solution (pH 11) after photoirradiation with visible-light in the presence of Rose Bengal (Abs_{530} 0.5). Inset I: absorption spectra of tyrosine (ca. 0.5 mM) in water, pH 11 (*a*) and in 0.01 M CTAC, pH 11 (*b*). Inset II: Absorption spectra of Rose Bengal in water, pH 11 (*a*) and in 0.01 M CTAC, pH 11 (*b*). Numbers on the main figure represent irradiation times (sec)

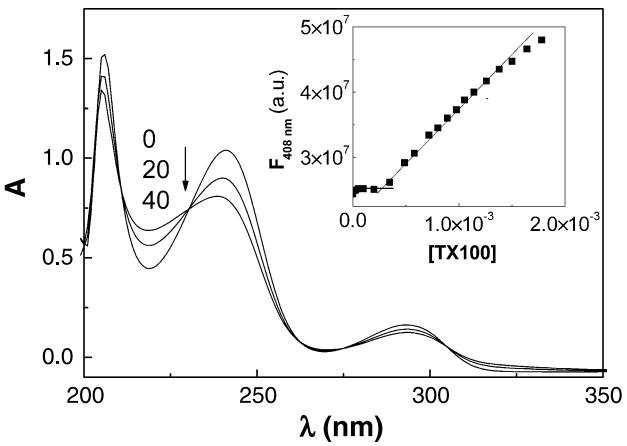

Fig. 2. Spectral changes of tyrosine-methyl ester 0.1 mM in air-equilibrated aqueous solution (pH 11) after photoirradiation with visible-light in the presence of Rose Bengal (Abs_{530} 0.5). Inset: Fluorescence intensity (F) of N-phenyl-1-naphthylamine in pH 11 aqueous solution, as a function of Triton X100 concentration, in the presence of tyrosine 0.5 mM. Numbers on the spectra represent irradiation times (sec)

oxygen, as well as in the presence of 10 mM NaN_3, a known selective $O_2(^1\Delta_g)$ quencher; b) the spectral changes in D_2O, at the same irradiation fluences, were comparatively faster than in H_2O, Foote (1984), Bertolotti et al. (1991), Miskoski et al. (1993), Criado et al. (1995) and Wilkinson et al. (1995). According to currently accepted criteria, these observations indicate that the photooxidation of tyr-D is largely an $O_2(^1\Delta_g)$-mediated process, Foote (1984). Furthermore, an unambiguous evidence for this interaction is given by the $O_2(^1\Delta_g)$ quenching experiments performed by TRPD (*vide infra*).

The following scheme summarizes the most important reactions of generation and deactivation of $O_2(^1\Delta_g)$, and were employed for the evaluation an discussion of the present results.

$$RB \xrightarrow{h\nu} {}^1RB^* \xrightarrow{k_{isc}} {}^3RB^* \quad (1)$$

$${}^3RB^* + O_2(^3\Sigma_g^-) \xrightarrow{k_{ET}} RB + O_2(^1\Delta_g) \quad (2)$$

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

$$O_2(^1\Delta_g) + \text{tyr-D} \xrightarrow{k_q} O_2(^3\Sigma_g^-) + \text{tyr-D} \quad (4)$$

$$O_2(^1\Delta_g) + \text{tyr-D} \xrightarrow{k_r} \text{products} \quad (5)$$

Scheme 1. Main steps in a sensitized photooxidation process, mediated by singlet molecular oxygen $[O_2(^1\Delta_g)]$

The absorption of light by RB, the sensitiser, gives rise to the electronically excited singlet and triplet states (reaction (1)), and an energy transfer from this triplet to ground state molecular oxygen $O_2(^3\Sigma_g^-)$ generates the excited oxygen species $O_2(^1\Delta_g)$ (reaction (2)). This can decay by light emission (phosphorescence) and by collision with surrounding molecules (typically the solvent) (reaction (3)), or interact physically (reaction (4)) or chemically (reaction (5)) with a quencher.

From the $O_2(^1\Delta_g)$ decay analysis at several tyr-D concentrations, the values of k_t ($k_t = k_q + k_r$) were determined (Table 1) employing TRPD from linear Stern-Volmer plots (Fig. 3). D_2O instead of H_2O was employed in the time-resolved experiments, in order to enlarge the $O_2(^1\Delta_g)$ lifetime to detectable limits of our apparatus, Nonell et al. (1995).

$$\tau_0/\tau = 1 + k_t\tau_0[\text{tyr-D}] \quad (6)$$

Using the specific oxygen electrode the rates of oxygen uptake by the photoirradiated tyr-D solutions were measured, in water, in CTAC and in TX100 solutions. Oxygen

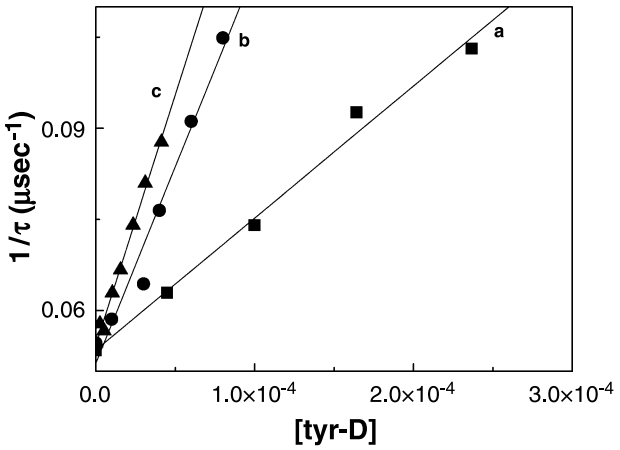

Fig. 3. Stern-Volmer plot for the quenching of $O_2(^1\Delta_g)$ phosphorescence (analytical wavelength 1270 nm) by: tyrosine in pH 11 water (*a*), tyrosine ethyl ester in pH 11 aqueous solution, in the presence of Triton X100 10 mM (*b*) and tyrosine methyl ester in pH 11 aqueous solution, in the presence of CTAC 10 mM (*c*). Rose Bengal (Abs_{532} 0.3) as a photosensitiser

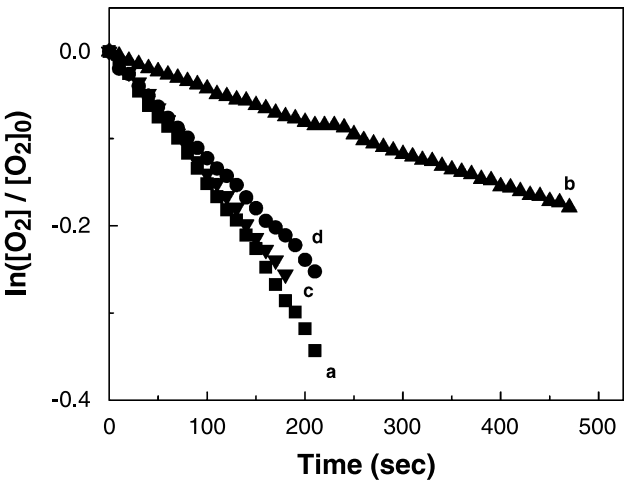

Fig. 4. First order plot of oxygen uptake in the Rose-Bengal-sensitized photooxidation of tyrosine methyl ester 0.5 mM in pH 11 water (*a*), in pH 11 CTAC 10 mM (*b*), in pH 11 Triton X100 10 mM (*c*) and of furfuryl alcohol 0.5 mM in pH 11 water (*d*). Rose Bengal as a photosensitiser (Abs_{530} 0.5)

consumption upon irradiation of aerated solutions of RB plus tyr-D was totally inhibited by 10 mM NaN_3.

The k_r values for tyr-D (Table 1), were graphically obtained through the described treatment of the data of oxygen uptake (Fig. 4).

Regarding $O_2(^1\Delta_g)$-mediated photooxidations (involving processes (9–11), their efficiency is correctly evaluated through the determination of the photooxidation quantum efficiency, $\varphi_r = k_r[\text{tyr-D}]/(k_q + k_t[\text{tyr-D}])$, García (1994). The φ_r value so determined takes into account the simultaneous effect of the physical and che-

Table 1. Rate constant values for the overall (k_t, M^{-1} sec^{-1}) and reactive (k_r, M^{-1} sec^{-1}) quenching of singlet molecular oxygen [$O_2(^1\Delta_g)$] by tyrosine and related compounds, and k_r/k_t ratios, in water, CTAC 10 mM and Triton X100 10 mM, all in pH 11 buffered solutions

tyr-D	k_r water $\times 10^8$ M^{-1} sec^{-1}	k_r TX100 $\times 10^8$ M^{-1} sec^{-1}	k_r CTAC $\times 10^6$ M^{-1} sec^{-1}	k_t water $\times 10^8$ M^{-1} sec^{-1}	k_t TX100 $\times 10^8$ M^{-1} sec^{-1}	k_t CTAC $\times 10^9$ M^{-1} sec^{-1}	k_r/k_t water	k_r/k_t TX100	k_r/k_t CTAC
tyr	1.5 ± 0.1	0.37 ± 0.01	6.1 ± 0.2	2.4 ± 0.1	3.5 ± 0.1	1.4 ± 0.1	0.6	0.1	0.004
tyrME	3.3 ± 0.2	1.4 ± 0.1	4.2 ± 0.1	4.9 ± 0.1	5.8 ± 0.1	0.82 ± 0.02	0.7	0.2	0.005
tyrEE	2.3 ± 0.1	1.3 ± 0.1	5.4 ± 0.2	2.3 ± 0.1	3.2 ± 0.1	4.4 ± 0.1	1	0.4	0.001
tyrBzE	0.042 ± 0.002	0.033 ± 0.002	1.2 ± 0.1	1.6 ± 0.1	1.8 ± 0.1	0.38 ± 0.01	0.03	0.02	0.003

mical interactions, being the k_q contribution usually interpreted in practical terms as a sort of *self-protection* against $O_2(^1\Delta_g)$-mediated photooxidations. Nevertheless, the φ_r value depends on the concentration of the photooxidizable substrate ([tyr-D] in the present case), which is particularly difficult to estimate in complex systems such as biological environments. On the other hand, no relevant information about these photoreactions can be obtained from the straightforward analysis of isolated k_t and k_r values, García (1994). A simpler and more useful approach is the evaluation of the k_r/k_t ratio (Table 1), which can be envisaged as the fraction of the overall interaction $O_2(^1\Delta_g)$-substrate that leads to effective chemical transformation. In other words, in a biological environment, a low k_r/k_t ratio would mean some degree of *self-protection* of a given substrate against photodynamic damage, and also a protection for surrounding photooxidizable targets. This, on the basis that a low k_r/k_t quotient indicates that the oxidative species is mainly physically deactivated by the substrate and that its reactive (oxidative) component is reduced to a lower extent.

Discussion

It is known that substrate solubilization in micelles can occur in two different sites: one corresponding to a hydrophobic region, in the micellar interior and the other, in a more polar environment, at the micelle-water interface, Bertolotti et al. (1992).

In previous papers we studied the interaction between cationic micelles and different phenols and hydroxybyphelys, Bertolotti et al. (1992), Biasutti et al. (1997) and Luiz et al. (2002). The results indicate that those phenolic compounds in their anionic forms interact with CTAB and CTAC micelles. This fact clearly obeys to an association between phenolate ions and the cationic head groups on the micelle, due to delocalisation of the charge in the phenolate. In this paper we assume for tyr-D a similar interaction with CTAC micelles that the above described for other phenols and hydroxyaromatic derivatives. Zhang and Xu (1994), reported on the photosensitizing properties of metal-phthalocyanines in pH 10 CTAC and aqueous solutions, employing tyr as a photooxidizable target. The main emphasis of the investigation was centered on the interaction sensitiser-micelle and on the competence between the pathways of electron abstraction and singlet oxygen generation, by the sensitizers. The authors also conclude that tyr is bound to the micellar surface in CTAC and intercalated into the interior of the micellar phase in TX100.

In our case, the changes in the electronic absorption spectra of tyr-D in the presence of CTAC micelles (Fig. 1, inset), clearly indicate an association substrate-micelle. On the other hand, the absence of any spectral shift for the system tyr-D – TX100, with respect to water as a solvent, strongly suggests an absence of interaction between tyr-D and the micelles. This result is in agreement with previous studies, Rossi et al. (1981), Encinas and Lissi (1982) and Lambert et al. (1986), in the sense that free amino acids are not incorporated into TX100 micelles. In our case the exception was tyrBzE, for which the little blue shift observed in TX100 could indicate that it is some included in the less-polar micellar region. This is possibly due to the enhanced hydrophobic character of the benzil ester moiety as compared to the other tyr-D herein studied.

Before discussing the kinetics of the $O_2(^1\Delta_g)$-mediated photooxidation of tyr-D in micellar solutions, some comments should be done with reference to the possible location of RB in the micellar environment. Our results (Fig. 1, inset II) show the red-shifted spectrum of the dye, associated to CTAC micelles, due to an electrostatic attraction between both the cationic micellar heads and the negatively charged RB. Besides, it is necessary to stress on the dynamics of $O_2(^1\Delta_g)$ in micellar media: regardless of their locus of generation, the molecules of the oxidative species effectively escape to the aqueous solution and visit several micelles, prior to deactivation Lissi et al. (1993), Bilski and Chignell (1994) carried out, in the past decade, a systematic and detailed study on the sensitising properties of RB dissolved in differently

$$O_2(^1\Delta_g) + \text{OHAD} \leftrightarrows [O_2(^1\Delta_g)\cdots\text{tyr-D}] \rightarrow \text{products}$$
$$\downarrow$$
$$[O_2(^3\Sigma_g^-)\cdots\text{tyr-D}] \rightarrow O_2(^3\Sigma_g^-) + \text{tyr-D}$$

Scheme 2. Quenching of singlet molecular oxygen $[O_2(^1\Delta_g)]$ by tyrosine- derivatives

charged and neutral micelles. The authors established that, irrespective of the micelle charge, micellised RB is more resistant to photobleaching than free RB and produce $O_2(^1\Delta_g)$ efficiently. This is consistent with our observations on the lack of the RB absorption spectrum changes after relatively prolonged photoirradiation.

Finally, in order to rationalize the kinetic results, the following consideration should be taken into account: it has been established that the process of $O_2(^1\Delta_g)$ quenching by phenolic compounds and N-Heteroaromatic hydroxy derivatives in general is mainly due to the presence of the OH group in the aromatic ring. It occurs through an encounter complex $[O_2(^1\Delta_g)\cdots\text{tyr-D}]$ (Scheme 2), possessing partial degree of charge-transfer character, being here tyr-D a generic OH-aromatic compound, Gorman et al. (1979), García (1994) and Pajares et al. (1998). As a consequence, the kinetics of the process of $O_2(^1\Delta_g)$ quenching is clearly favoured by an increase in the solvent polarity.

According to the former mechanism, both chemical reaction (generation tyr-D oxidation products) and physical quenching (regeneration of $O_2(^3\Sigma_g^-)$, without chemical change in tyr-D) can operate, and the relative importance of each process is reflected by the values of the respective rate constants k_r and k_q.

From the kinetic data in Table 1, the following remarkable points arise:

a) The higher overall $O_2(^1\Delta_g)$ – tyr-D interaction, represented by the respective k_t values, were obtained in CTAC, whereas the corresponding values in water and in TX100 are very similar each other, although scarcely higher in TX100.
b) k_r values smoothly decrease going from water to TX100 and abruptly decrease going from water to the CTAC micellar system.
c) k_r/k_t values, increase in the sense CTAC$\ll$TX100$<$ water, indicating, for the tyr-D family studied, an excellent degree of *self-protection* against $O_2(^1\Delta_g)$-attack in the CTAC micellar system and a high photooxidability level in water. TX100 micellar medium represents an intermediate situation.
d) tyrBzE constitutes an exception to some of the former generalizations: its k_r values in water and in both micellar systems are low and not so different each other, whereas the k_r/k_t value remains practically the lower in all three media.

Regarding the increase in k_t values going from water to the CTAC micellised medium could be explained by the marked change in polarity of the medium. At pH 11 the substrate occupies the highly polar water-CTAC micelle interfacial region. The relatively small increase in k_t values, going from water to TX100 could obey to an increase of tyr-D local concentration of the substrate in the interfacial region of the micelle This behaviour has already been reported for N-methyl phenothiazine-oil/water emulsions; whereas the simultaneous smooth decrease in respective k_t values could be due to a small reduction in the polarity of the environment by the presence of micellised TX100, Braun and Oliveros (1991).

The respective k_r values of tyr-D in CTAC, as compared to those in water, appear to be mainly affected by the electron-donor abilities of the tyr-D. The electrostatic interaction of the polar heads of the micelles with the ionized tyr-D decreases the electron-donor capabilities of the phenoxy groups.

The k_r value for tyrBzE in water (Table 1) is between 40- and 80-times lower than the corresponding values for tyr, tyrME and tyrEE. Also, this value is atypically low for phenolic compounds in the alkaline pH range, Wilkinson et al. (1995). A possible explanation for this behaviour is related to an inefficient reactive process in the benzyl-substituted compound due to the nature of entrophy-controlled step of the reactive pathway within the encounter complex (Scheme 2), as previously postulated for indoles, furans and some phenolic derivatives such as α-tocopherol, Gorman et al. (1979, 1984). The reactive pathway is affected irrespective to the nature of the reaction medium involved. Simultaneously, k_t values for tyrBzE are relatively high in all media studied, as corresponds to a reaction controlled by solvent polarity and local concentration of the substrate. As a consequence, the respective k_r/k_t values indicate very low photodegradation efficiencies for the benzyl-derivative.

Finally, a comparison between the evolution of k_t and k_r/k_t values in water and in CTAC and TX100 micellar systems indicate that tyr-D in CTAC micelles behaves as the most efficient $O_2(^1\Delta_g)$ scavengers. In this sense, they exhibit a better degree of *self-protection* against $O_2(^1\Delta_g)$-mediated photooxidation with the highest values for the overall quenching rate constant and simultaneously the lowest k_r/k_t ratios. In other words this mean that this combination tyr-D/medium offers the greatest deactiva-

tion of the photooxidative species $O_2(^1\Delta_g)$, and a minimal substrate loss, whereas water is a medium that favours photodegradation, with photooxidation efficiencies between 100 and 60% (Table 1).

Conclusions

Results on the kinetics of tyr-D sensitized photooxidation are interpreted in terms of a competition between solvent polarity effects, local substrate concentration and electron donating capabilities of the substrates in the different media. Nevertheless, it should be emphasized that although all the conditioning factors could be simultaneously present in a given system, such as an environmentally complex biological medium, the prevalence of one of them determines the photodegradative fate of a substrate. In this context, the knowledge of the photooxidative kinetic behaviour of AAs residues, under given experimental conditions, can contribute to predict the extent of photodynamic damage in biological environments.

Acknowledgements

Financial support from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Secretarías de Ciencia y Técnica from Universidad Nacional de Río Cuarto (SECyT UNRC), and from Universidad Nacional de la Patagonia Austral (SECyT) UNPA is grateful acknowledged.

References

Amat-Guerri F, López-González MMC, Martínez-Utrilla R, Sastre R (1990) Singlet oxygen photogeneration by ionized and un-ionized derivatives of Rose Bengal and Eosin Y in diluted solutions. J Photochem Photobiol A Chem 53: 199–210

Bertolotti SG, García NA, Gsponer HE (1989a) Micellar binding of polychlorophenols. J Colloid Interface Sci 129: 406–413

Bertolotti SG, Gsponer HE, García NA (1989b) Surfactant effect on the sensitized photooxidation of polychlorophenlic pesticides. Toxicol Environ Chem 22: 229–237

Bertolotti SG, Argüello GA, García NA (1991) Effect of the peptide bond on the singlet molecular oxygen mediated photooxidation of tyrosine and tryptophan dipeptides. A kinetic study. J Photochem Photobiol B Biology 10: 57–70

Bertolotti SG, Gutiérrez MI, Bocco G, Bertolotti SG, García NA (1992) Sensitized photooxygenation of mononitrophenols in micellar media. Toxicol Environ Chem 35: 115–123

Biasutti MA, Bertolotti SG, Soltermann AT, García NA (1997) Singlet molecular oxygen production and quenching by hydroxybiphenyls in micellar media. Toxicol Environ Chem 64: 1–13

Bilski P, Chignell CF (1994) Properties of differently charged micelles containing Rose Bengal: application to photosensitization studies. J Photochem Photobiol A Chem 77: 49–58

Braun A, Oliveros E (1991) Oxidation reactions in microheterogeneous media. In: Scheider H-J, Dürr H (eds) Frontiers in supramolecular organic chemistry and photochemistry. VCH Wiley, Weinheim, pp 393–419

Chattopadhyay A, Mukherjee S, Raghuraman H (2002) Reverse micellar organization and dynamics: a wavelength-selective fluorescence approach. J Phys Chem B 106: 13002–13009

Criado S, Soltermann AT, García NA (1995) Influence of the nuclear and extranuclear substitution on the singlet molecular oxygen $O_2(^1\Delta_g)$-mediated photooxidation of tyrosine derivatives. A kinetic study. Amino Acids 8: 367–377

Criado S, Bertolotti SG, Soltermann AT, García NA (1997) Kinetic studies on the photosensitized oxidation [$O_2(^1\Delta_g)$-mediated] of tryptophan-alkyl-esters in Triton X-100 micellar solutions. J Photochem Photobiol B Biol 38: 107–113

Criado S, Soltermann AT, Marioli JM, García NA (1998) Sensitized photooxidation of di- and tri-peptides of tyrosine. Photochem Photobiol 68: 453–458

Criado S, Marioli JM, Allegretti PE, Furlong J, Mártire D, García NA (2001) Oxidation of di- and tri-peptides of tyrosine and valine mediated by singlet molecular oxygen, phosphate radicals and sulfate radicals. J Photochem Photobiol B Biol 65: 74–84

Encinas MV, Lissi EA (1982) Evaluation of partition constants in compartamentalized systems from fluorescence quenching data. Chem Phys Lett 91: 55–57

Fendler JH (1982) Membrane mimetic chemistry, Chapter 2. Wiley, New York, pp 6–47

Foote CS (1984) Mechanisms of photooxidation. In: Doiron DG, Gomer CJ (eds) Porphyrin localization and treatment of tumors. Alan R. Liss Inc, New York, pp 3–18

García NA (1994) Singlet molecular oxygen mediated photodegradation of aquatic phenolic pollutants. A kinetic and mechanistic overview. J Photochem Photobiol B Biol 22: 185–196

Gorman AA, Lovering G, Rodgers MAJ (1979) The entrophy controlled reactivity of singlet oxygen $O_2(^1\Delta_g)$ toward furans and indoles in toluene. A variable temperature study by pulse radiolysis. J Am Chem Soc 101: 3050–3054

Gorman AA, Gould IR, Hamblett I, Standen MC (1984) Reversible exciplex formation between singlet oxygen $O_2(^1\Delta_g)$ and vitamin E. Solvent and temperature effects. J Am Chem Soc 106: 6956–6959

Lambert CR, Reddi E, Spikes JD, Rodgers MAJ, Jori G (1986) The effects of porphyrin structure and aggregation state on photosensitized processes in aqueous micellar media. Photochem Photobiol 44: 595–601

Lissi EA, Encinas MV, Lemp E, Rubio MA (1993) Singlet oxygen $O_2(^1\Delta_g)$ bimolecular processes. Solvente and compartamentalization effects. Chem Rev 93: 699–724

Luiz M, Biasutti A, García NA (2002) Micellar effect on the scavenging of singlet molecular oxygen by hydroxybenzenes. Redox Report 7: 23–28

Michaeli A, Feitelson J (1994) Reactivity of siglet oxygen toward amino acids and peptides. Photochem Photobiol 59: 284–289

Miskoski S, Bertolotti SG, García NA, Argüello G (1993) On the singlet-molecular oxygen-mediated photooxidative behaviour of the tripeptide glycyl-tyrosyl-alanine in alkaline medium. A kinetic study. Amino Acids 4: 101–110

Montfort C, Fernau NS, Beier JI, Sies H, Klotz L-O (2006) Extracellular generation of hydrogen peroxide is responsible for activation of EGF receptor by ultraviolet A radiation. Free Radic Biol Med 41: 1478–1487

Neumann M, García NA (1992) Kinetics of mechanism of the light-induced deterioration of lemon oil. J Agric Food Chem 40: 957–960

Nonell S, Moncayo L, Trull F, Amat-Guerri F, Lissi EA, Soltermann AT, Criado S, Garcia NA (1995) Solvent influence on the kinetics of the photodynamic degradation of the trolox, a water-soluble model compound for vitamin E. J Photochem Photobiol B Biol 29: 157–162

Pajares A, Gianotti J, Haggi E, Stettler G, Amat-Guerri F, Criado S, Miskoski S, García NA (1998) Kinetic study of the sensitized photo-

degradation of monohydroxylated *N*-heteroaromatic compounds. J Photochem Photobiol A Chem 119: 9–14

Parker NR, Jamie JF, Davies MJ, Truscott RJW (2004) Protein-bound kynurenine is a photosensitizer of oxidative damage. Free Radic Biol Med 37: 1479–1489

Scully FE, Hoigne J (1987) Rate constants for reactions of singlet oxygen with phenols. and other compounds in water. Chemosphere 16: 681–694

Soltermann AT, Biasutti MA, Senz A, García NA (1995) Influence of the pH on the photodynamic effect in lysozime. A comparative study with sensitized photooxidation of isolated amino acids. Amino Acids 9: 123–134

Straight RC, Spikes JD (1985) Photosensitized oxidation of biomolecules. In: Frimer AA (eds) Singlet oxygen, Chapter 2, vol. IV. CRC Press, Boca Raton, pp 91–143

Svistunenko DA (2005) Reaction of haem containing proteins and enzymes with hydroperoxides: The radical view. Biochim Biophys Acta Bioenerg 1707: 127–155

Weast RC (ed) (1963) Handbook of chemistry and physics, 55th ed. CRC Press, Boca Raton, pp 1974–1975

Wilkinson F, Helman W, Ross AB (1995) Rate constants for the decay of the lowest electronically excited singlet state molecular oxygen in solution. An expanded and revised compilation. J Phys Chem Ref Data 24: 663–1021

Authors' address: Norman A. García, Departamento de Química, Universidad Nacional de Río Cuarto, 5800 Río Cuarto, Argentina,
Fax: +54-358 4676233, E-mail: ngarcia@exa.unrc.edu.ar