Journal of Photochemistry and Photobiology A: Chemistry 209 (2010) 89–94

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

Visible-light promoted photoprocesses on aqueous gallic acid in the presence of riboflavin. Kinetics and mechanism

Adriana Pajares[a], Mabel Bregliani[a], M. Paulina Montaña[b], Susana Criado[c], Walter Massad[c], José Gianotti[a], Isela Gutiérrez[d], Norman A. García[c,*]

[a] Unidad Académica Río Gallegos, Universidad Nacional de la Patagonia Austral, 8400 Río Gallegos, Argentina
[b] Area de Química-Física, Universidad Nacional de San Luis, Lavalle 1151, 5700 San Luis, Argentina
[c] Departamento de Química, Universidad Nacional de Río Cuarto, 5800 Río Cuarto, Argentina
[d] Departamento de Química, Facultad de Ciencias Naturales, Universidad Nacional de la Patagonia SJB, 9000 Comodoro Rivadavia, Argentina

ARTICLE INFO

Article history:
Received 28 July 2009
Received in revised form 5 October 2009
Accepted 24 October 2009
Available online 31 October 2009

Keywords:
Gallic acid
Hydrogen peroxide
Photodegradation
Riboflavin
Singlet molecular oxygen
Superoxide radical anion

ABSTRACT

Within the frame of possible precursory photoreactions in the generation of humic substances, the visible-light promoted interaction between riboflavin (Rf), a native photosensitizer in aqueous systems, and gallic acid (GA), a polyphenol naturally formed after lignin degradation, was investigated. A systematic kinetic and mechanistic study was conducted under aerobic conditions in aqueous media, through visible-light continuous photolysis, polarographic detection of oxygen uptake, stationary and time resolved fluorescence spectroscopy, time resolved near-IR phosphorescence detection and laser flash photolysis techniques. GA is degraded relatively fast in pH 7 aqueous solutions, where singlet molecular oxygen ($O_2(^1\Delta_g)$), superoxide radical anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) – all three species photogenerated from triplet excited Rf – participate in the photoprocess. The general conclusion is that in natural waters GA can undergo spontaneous phototodegradation under environmental conditions. Radical species generated in the presence of Rf can participate in condensation or polymerization reactions promoting the natural synthesis of humic products.

1. Introduction

Humic acids (HA) play a dominant role in conditioning soil properties. They are species of relatively high molecular weight with several functional groups, predominantly carboxylic and phenolic ones [1]. Although the precise reaction sequence of natural HA synthesis is uncertain, apparently the oxidative polymerization of polyphenols in waters and soils is thought to be among the major processes of formation of natural humic substances [2]. Recent studies relating polyphenol content in olive-mill composting and lignin content suggest that polyphenols contribute to the synthesis of humic substances [3]. In fact, very recently was synthesized a water soluble humic-acid-like polycondensate, which mimics fundamental physicochemical and spectroscopic properties of natural humic acids [4]. The polymer was produced under oxygenated ambient by oxidative co-polymerization of gallic acid (GA) and protocatechuic acid. On these grounds, the knowledge of different natural oxidation channels and the generation of potentially reactive radicals that could operate in the early steps of carboxylic polyphenols oxidation may be of importance in order to understand the HA generation, under environmental conditions.

Many papers have been published on polyhydroxybenzenes and particularly on GA oxidation, through photochemical, enzymatic and thermal reactions [5–11]. GA abounds in nature since it is among the low molecular weight phenolic acids formed after lignin degradation [12]. In natural waters, the colorless GA could be oxidized under solar irradiation if some compounds, named photosensitizers, able to absorb visible light and to generate reactive excited or reactive oxygenated species (ROS), are present in the same aqueous environment. Several dissolved organic compounds usually present in natural aqueous media can act as solar photosensitizers [13]. Riboflavin (Rf, vitamin B_2), a well-known natural pigment present as traces in waters of rivers, lakes and seas [14,15] has been repeatedly postulated as a possible sensitizer for the photooxidative degradation of natural substrates and contaminants [16] and as a photoinitiator of polymerization in aqueous media [17]. The mechanism of sensitization with Rf is rather complex, in many cases with the concurrent involvement of the ROS $O_2(^1\Delta_g)$ and $O_2^{\bullet-}$, both generated with quantum yields of 0.49 and 0.009, respectively [18].

The aim of the present work was to carry out a kinetic and mechanistic study on the photoprocesses that operate on the polyhydroxylaromatic compound GA, under conditions fre-

---

* Corresponding author. Tel.: +54 358 4676439; fax: +54 358 4676233.
E-mail address: ngarcia@exa.unrc.edu.ar (N.A. García).

1010-6030/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotochem.2009.10.011

quently found in nature: an aqueous environment, in the presence of the native photosensitizer Rf, and under visible-light illumination. This knowledge could contribute to the understanding of possible mechanistic pathways for the initiation of condensations or polymerization reactions involving GA and other polyphenols in the medium, promoting the generation of humic substances.

2. Materials and methods

2.1. Materials

Riboflavin (Rf), deuterium oxide 99.9% (D_2O), superoxide dismutase (SOD) from boverythrocytes and gallic acid (GA), pyrogallol (PG), sodium azide (NaN_3) and catalase from bovine liver (CAT) were purchased from Sigma Chem. Co. Rose Bengal (RB), perinaphtenone (PN) and furfuryl alcohol (FFA) were from Aldrich. Hydrogen peroxide (H_2O_2), 100 vol. was from Parsol (Argentine). D_2O was employed in the time resolved determinations of $O_2(^1\Delta_g)$ in order to enlarge the lifetime of this species [16]. Water was triply distilled. Methanol (MeOH), HPLC quality, was provided by Sintorgan (Argentine).

The pH of the final aqueous solutions (Rf + GA or Rf + PG, in water) for all photochemical experiments was in the range 7 ± 0.2, employing buffered aqueous solutions prepared, with KH_2PO_4 0.025 M/Na_2HPO_4 0.025 M [19]. The presence of the salts in the mentioned concentrations did not affect neither the lifetimes nor the profiles of the optical spectra of Rf electronically excited states, as compared to those obtained in pure water.

2.2. Absorption and fluorescence measurements

Ground state absorption spectra were registered employing a Hewlett-Packard 8452A diode array spectrophotometer. Fluorescence lifetimes were determined with a time-correlated single photon counting technique (SPC) on an Edinburgh FL-9000CD instrument. Excitation and emission wavelengths for Rf were 445 and 515 nm, respectively. A classical Stern–Volmer treatment of the data was applied through Eq. (1), where $^1\tau$ and $^1\tau_0$ are the respective fluorescence lifetimes of Rf in the presence and in the absence of GA, and ^1k_q is the rate constant of the quenching of excited singlet Rf ($^1Rf^*$) by GA:

$$\frac{^1\tau_0}{^1\tau} = 1 + {}^1k_q{}^1\tau_0[GA] \quad (1)$$

2.3. Laser flash photolysis experiments

Argon-saturated 0.04 mM Rf aqueous solutions were photolysed using a flash photolysis apparatus. A ns Nd:Yag laser system (Spectron) at 355 nm was the excitation source, employing a 150 W Xenon lamp as analyzing light. The detection system comprised a PTI monochromator and a red-extended photomultiplier (Hamamatsu R666). The signal, acquired and averaged by a digital oscilloscope (Hewlett-Packard 54504A), was transferred to a PC via a Hewlett-Packard Interface Bus (HPIB), where it was analyzed and stored.

Triplet Rf ($^3Rf^*$) was generated by a 355 nm laser pulse, and its disappearance was monitored from the first order decay of the absorbance at 670 nm, a zone where the interference from other possible species is negligible. The triplet decay was measured at low Rf concentration (typically 0.05 mM) and at low enough laser energy, to avoid self-quenching and triplet–triplet annihilation. The rate constant for the interaction $^3Rf^*$–GA (^3k_q, reaction (7) in Scheme 1) was determined from a Stern–Volmer treatment (Eq. (2)):

$$\frac{^3\tau_0}{^3\tau} = 1 + {}^3k_q{}^3\tau_0[GA] \quad (2)$$

where $^3\tau$ and $^3\tau_0$ are the experimentally determined lifetimes of $^3Rf^*$ in the presence and in the absence of a GA, respectively.

2.4. Time resolved $O_2(^1\Delta_g)$ phosphorescence detection (TRPD)

The total quenching rate constant (k_t, see Scheme 1) for $O_2(^1\Delta_g)$ deactivation by GA, was graphically determined by near-IR time resolved phosphorescence, employing Eq. (2), being τ and τ_0 the respective $O_2(^1\Delta_g)$ lifetimes in the presence and in the absence of GA.

$$\frac{\tau_0}{\tau} = 1 + k_t\tau_0\,[GA] \quad (3)$$

The third harmonic ($\lambda = 355$ nm) from a Nd:Yag laser (Spectron) was used as the excitation source. The emitted ($O_2(^1\Delta_g)$) phosphorescence at 1270 nm was detected at right angles using a Edinburgh EI-P Germanium detector, after having passed through 1270 nm-interference and two wratten filters. The output of the detector was coupled to a 400 MHz digital oscilloscope (HP 54504A) and to a personal computer to carry out the signal processing. Usually, 10 shots were needed for averaging so as to achieve a good signal to noise ratio, from which the decay curve was obtained. Air equilibrated solutions were employed in all cases.

2.5. Stationary photolysis and oxygen uptake experiments

Stationary aerobic photolysis of aqueous solutions containing typically 0.2–0.5 mM GA or PG and 0.04 mM Rf was carried out in a PTI unit, provided with a high pass monochromator and 150 W Xe lamp, irradiating with 445 ± 10 nm, or in a home-made photolyser for non-monochromatic irradiation (150 W quartz-halogen lamp). In this case cut-off filters (360 nm) ensured that the light was only absorbed by the sensitizer.

The Rf-sensitized photooxygenation rates of 0.5 mM GA and PG and 0.02 mM Rf were determined by evaluation of the initial slopes of oxygen consumption *vs.* irradiation time, employing a specific oxygen electrode (Orion 97-08).

Oxygen uptake in water was monitored with a 97-08 Orion electrode. Assuming that the reaction of RB-generated $O_2(^1\Delta_g)$ with the quencher is the only way of oxygen consumption, the ratio of the first order slopes of oxygen uptake by GA, each at the same concentration, yields k_r/k_{rR}. The reference was FFA, with a reported pH-independent k_r value of $1.2 \times 10^8\ M^{-1}\,s^{-1}$ [20].

3. Results

3.1. The sensitized photooxidation process

The main photoinduced processes that take place when a solution containing GA and Rf is irradiated with visible light in the presence of oxygen, are shown in Scheme 1. These processes include photoinduced reactions, both in the presence and in the absence of a substrate, the prevalence of which usually depends on the experimental conditions and on the involved compounds GA and Rf. This self-explanatory Scheme has been previously discussed in regard with similar processes applied to other substrates [16]. P(n) represent different photoproducts. The reported pK values for GA are 4.21 and 8.54 and, respectively correspond to the acidic ionization of carboxylic group and the ionization of the first phenolic group [21]. Hence, in the following the symbol GA will represent the monoanionic form of gallic acid, the species present