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Full Length Article

Photophysical properties of neutral and dissociated forms of rosmarinic acid

Nader Al Danaf [a,b], Racha Abi Melhem [a], Khaleel I. Assaf [b], Werner M. Nau [b], Digambara Patra [a,*]

[a] Department of Chemistry, American University of Beirut, Beirut, Lebanon
[b] Department of Life Sciences and Chemistry, Jacobs University Bremen, Bremen, Germany

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ABSTRACT

Polyphenols are bioactive components that have attracted attention by chemists over many years. Rosmarinic acid (RA) is a polyphenol that is widely investigated for its broad range of potential applications as an anti-carcinogenic agent, an anti-inflammatory drug, and antioxidant. The spectroscopic properties of this molecule are addressed in details in this study. The absorption and fluorescence of RA are investigated at different pH values, characterizing the dissociated forms of RA. In a similar manner, RA was characterized in a set of different solvents. The spectral shifts of RA in the different solvents were addressed by using the Lippert–Mataga and the Stokes' shift *vs.* E_T30 plots, which revealed two sets of linearity for the behavior of RA in polar protic and aprotic solvents. The lifetime decay profile of the dissociated forms of RA and those of RA in different solvents are characterized by bi-exponential lifetime decay functions. A theoretical study on the molecular structure of the different dissociated forms of RA is also reported. The simulated UV–vis spectra of the dissociated forms of RA were studied and compared to the experimental spectra by performing TDDFT calculations. Besides the UV-simulated spectra, the frontier molecular orbitals (FMO) of the different RA dissociated forms were also calculated.

1. Introduction

Polyphenols are of growing interest not only as natural and/or dietary sources but also due to their large spectrum of medicinal, pharmaceutical, and biological effects [1–3]. Their photophysical and photochemical behavior is also of great importance in regard to potential medicinal applications [4–6]. Various polyphenols have been studied with respect to their antioxidant and anti-carcinogenic effects [7,8]. Among these, rosmarinic acid (RA) has been subject of numerous investigations in the areas of biology, medicine, and pharmacology [9,10]. The profound effects of RA on human health includes antioxidant [11,12], astringent [9,13], anti-inflammatory [14–16], anti-mutagen [17], anti-bacterial [18], anti-viral [19], anti-amyloid [20], and potent anti-HIV and anti-tumor [21,22] activities. RA, a natural phenolic compound, is an ester of caffeic acid and 3,4-dihydroxyphenyl lactic acid. It is found in *Lamiaceae* and *Boraginaceae* family plants, including rosemary, basils, thyme, sage and, oregano [9]. Thus, RA has become a valuable product in the food, cosmetics, and pharmaceutical industry [23]. As a phenolic compound, RA is significant as it directly contributes to antioxidant activity [11,12,24–26].

The photophysical properties of polyphenols have been reported earlier with a specific focus on the optical properties of such polyphenolic systems, which have been found to be strongly dependent on the nature of the solvent and on the pH of the solution. Favaro et al. have studied the acido- and iono-chromic properties of apigenin and luteolin as an important step that is responsible for the color changes with aging, which is of particular interest in diagnostics and for the conservation of art work [27]. The structural and acid-base properties of luteolin and apigenin have also been reported [27]. TDDFT calculations have been used to investigate and analyze several aspects of apigenin and luteolin flavonoids, which allowed correlations of experimental optical properties to theoretically computed ones [28]. The optical absorption and fluorescence properties of fisetin, another polyphenol belonging to the flavonoid group, have been found to show two emission peaks in organized media such as liposomes [29,30], bile salt media [31], and cyclodextrins [32]. Another widely investigated natural phenol, curcumin, is well known for its anti-oxidant, anti-inflammatory, anti-cancer, and anti-amyloid activities [33–35]. The photophysical properties and solvation dynamics of curcumin, including its excited state and dissociated forms, have been widely investigated [36–38], because photophysical

* Corresponding author.
E-mail address: dp03@aub.edu.lb (D. Patra).

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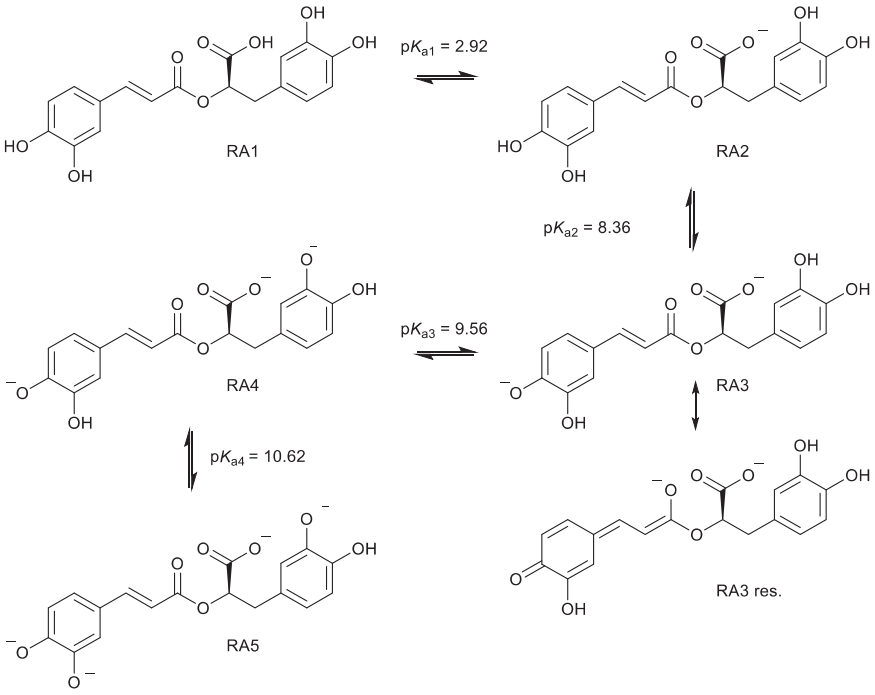

Scheme 1. Chemical structures of the neutral and dissociated forms of RA. pK_a values were taken from Ref. [57].

phenomena are closely associated with the medicinal properties of pigment molecules such as hypericin and hypocrelin [39–42]. The photophysical properties of curcumin and its different dissociated forms have been described theoretically as well [43]. Ultrafast fluorescence up conversion studies have been used to address this issue. It was found that H-atom transfer is the preferred antioxidant mechanism of curcumin [44].

Despite the large number of experimental studies on the antioxidant properties of RA, its absorption, fluorescence, and excited-state properties, which are crucial to understand its antioxidant activity, have not been reported in detail. The mechanism of phenolic antioxidants is believed to be the scavenging or quenching of free radicals and reactive oxygen species [45–47]. DFT investigations on the antioxidant activity of RA have also been performed [48]. Numerous methods have been applied for the analytical determination of RA by using spectrophotometric methods [49–52]. Recently, Mariappan et al. have theoretically studied the IR and Raman spectroscopy spectra of RA [53]. In this paper, we combined experimental and theoretical methods to study the photophysical properties of RA and its dissociated forms.

2. Experimental

2.1. Materials

RA was purchased from Sigma-Aldrich and used as received. The solvents used were of spectroscopic grade and obtained from Sigma-Aldrich. The solutions were equilibrated for 30 min before taking any measurement.

2.2. Spectroscopic measurements

Absorption spectra were recorded at room temperature on a Varian Cary 4000 UV–vis Spectrophotometer. The steady-state fluorescence (emission and excitation) measurements were recorded with a 1-nm resolution increment, 2.5-nm excitation, and 5-nm emission slits on a JASCO FP-8500 spectrofluorometer. The fluorescence lifetime measurements were performed by time-correlated single-photon counting with a FLS920 lifetime spectrometer (Edinburgh Instruments). A pulsed LED (PicoQuant, PLS-280, fwhm ca. 300 ps) was used for excitation at 297 nm. The instrument response function was recorded routinely and used for deconvolution. The average fluorescence lifetime (τ_{avg}) values were obtained by using the equation below:

$$\tau_{avg} = \left(\sum_{i=1}^{n} \alpha_i \tau_i^2\right) \Big/ \left(\sum_{i=1}^{n} \alpha_i \tau_i\right)$$

where τ_i were the individual lifetimes with the corresponding amplitudes α_i.

2.3. Computational details

All calculations were performed with the Gaussian 09 [54]. The ground-state geometries were optimized using density-functional theory, DFT. The Becke3-Lee–Yang–Parr hybrid functional (B3LYP) method was used for the full optimization with 6-31+G(d,p) basis set. [55,56]. Minima of the calculated structures were characterized by the absence of imaginary frequencies. For each dissociated form of RA, all possibilities were taken into consideration taking only the most stable one for further calculations. The calculations for the HOMO and LUMO frontier orbital energies were obtained using Time-Dependent-DFT (TD-DFT) at the B3LYP/6-31+G(d,p) level of theory. The effect of solvation (water as a solvent) was taken into consideration by means of the self-consistent reaction-field (SRCF) method, based on SMD solvation model implemented in Gaussian 09.

3. Results and discussion

3.1. Effect of pH

All possible protonation states for RA are shown in Scheme 1. The pH-dependent absorption and fluorescence spectra of RA are presented in Fig. 1. The absorption spectrum of RA1 at pH 1.1 showed a main peak at 328 nm and a shoulder at 288 nm