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Photophysicochemical characterization of mycosporine-like amino acids in micellar solutions

D. E. Orallo, [a] S. G. Bertolotti [b] and M. S. Churio *[a]

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The properties and photochemical and photophysical behavior of the mycosporine-like amino acids (MAAs) shinorine and porphyra-334 were experimentally evaluated in solutions of direct ionic micelles as simple biomimicking environments. The preferential partition of the natural molecules in the aqueous phase of sodium dodecyl sulfate (SDS) or cetyltrimethylammonium chloride (CTAC) micellar systems is confirmed. Although the proton dissociation of the carboxylic groups in the MAAs is slightly inhibited in CTAC solutions, the molecules are predicted to be in the form of zwitterions in all the explored media around physiological pH. The increase in the fluorescence quantum yield, emission lifetime and stationary anisotropy in the presence of CTAC micelles suggest electrostatic attractions of the MAAs with the surface of the cationic micelles. Consistently, the triplet–triplet absorption spectra in CTAC solutions reveal the typical environmental features of the micellar interface, while in the presence of SDS they are similar to those determined in neat water. Finally, the photostability of the MAAs increases in the micellar systems, more noticeably in the case of CTAC. It is concluded that the ability of the two MAAs to act as UV screens is susceptible to the influence of electrostatic interactions with organized microheterogeneous environments.

Introduction

Since stratospheric ozone depletion became evident, the effects of UV radiation on the environment and the development of new materials for improved UV-photoprotection became of great concern. Within this context, the cosmetics and pharmaceutical industries find in natural products good alternatives to synthetic compounds as new approaches for photoprotection and sunscreen formulations.[1,2] In particular, substances of marine origin represent a promising resource for varied innovative biotechnological developments.[3–5] These usually involve the design of an appropriate management strategy that typically requires solubilization and vehiculization through microheterogeneous systems.[6,7] Moreover, the encapsulation or combination of active molecules with nanoparticles has been widely explored lately, generally providing enhanced photoprotective effectiveness in different ways, such as delivery control, increasing the absorption or scattering of UV radiation, reducing toxicity, or improving the stability of the sunscreens.[8,9] Still the problem of the safety of complex ingredient mixtures remains, and it is particularly important in the development of suncare preparations that requires minimization of the risks of phototoxic responses.[10]

Marine metabolites such as mycosporine-like amino acids (MAAs) and gadusols have attracted great interest in relation to their photoprotective potential. They are water soluble small molecules with high molar absorption coefficients in the UV-A and/or UV-B regions. Although they are synthesized by algae, fungi and bacteria, they can also be found in a wide variety of organisms.[11,12]

Experimental and theoretical studies on various MAAs and their related structures have confirmed their high photochemical robustness in solution. This feature is explained in terms of the rapid relaxation of the excited electronic states by efficient dissipation of the absorbed UV radiation energy as heat.[13,14] However, detailed physicochemical, photophysical and photochemical properties of this family of molecules in complex biological environments have been less described.

In nature, some MAAs can be found in close association with a mineral phase in marine diatom frustules, which it has been suggested could further stabilize the metabolites, thereby enhancing their photoprotection ability against UV.[15] In addition, grafting of MAAs to a biopolymeric matrix of chitosan has been recently reported by Fernandes *et al.*[16] They have proved that the photostability of the resulting conjugates is

[a]*IFIMAR, Instituto de Investigaciones Físicas de Mar del Plata (CONICET-UNMDP), Departamento de Química, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Funes 3350, (B7602AYL) Mar del Plata, Argentina. E-mail: schurio@mdp.edu.ar*
[b]*Departamento de Química, Universidad Nacional de Río Cuarto, CONICET, (X5804BYA) Río Cuarto, Argentina*

preserved. In addition, the synthesis of conjugated ZnO nanoparticles with the MAA shinorine mediated by cyanobacteria has been observed, but no evidence of the mechanism for the conjugate formation was revealed.[17]

Micelles are among the simplest biomimetic microenvironments investigated in various areas of biology, biochemistry and medicine. Typically, the dynamics of the excited states of a given substrate can vary according to the portion of the micelle in which it is preferentially solubilized. The behavior is usually determined by the species distribution among hydrophilic and hydrophobic domains, and general or specific interactions with the microenvironment.[18,19]

In this scenario, it is important to note that the definite chemical structure of some MAAs has been a matter of discussion. The question of which form, the protonated or the neutral, is relevant for the activity of the compounds *in vivo* has been addressed. For instance, the zwitterionic character of MAAs has been invoked to rationalize the influence of pH on the oxidation feasibility of a series of MAAs isolated from red macroalgae.[20] Klisch *et al.* applied NMR techniques and *ab initio* calculations to explore the stereo structure of porphyra-334 and found the best agreement for the imino *N*-protonated form. In addition, an exceptionally large proton affinity became evident from the calculations and, for this reason, the molecule was described as a "proton sponge".[21] Moreover, Matsuyama *et al.* claim in a recent study that charge resonance conjugation in the protonated forms of the 3-aminocyclohexenone or 1-amino-3-iminocyclohexene moieties is the origin of the UV-protective functions of aqueous MAAs.[22] However, this concept is weakly supported and overlooks the central role of conical intersection points between the excited and ground singlet state potential energy surfaces. According to a computational study on palythine as a model MAA, the protonated imino species is responsible for the strong UV absorption, although the fact that the photochemical fates of both neutral and protonated forms coincide in their rapid non radiative deactivation has been empirically verified.[23–25]

Altogether, it is clear that MAAs may not appear as neutral species in solution. Thus, one should expect that interactions between MAAs and charged micellar media may influence their bioactivity, so the evaluation of these basic features is important to fully understand the variables that control the photoprotective potential of these metabolites in different media.

In this work, we report on an experimental study of the physicochemical and photophysicochemical properties of the MAAs shinorine and porphyra-334 in the presence of anionic and cationic direct micelles, prepared with sodium dodecyl sulfate (SDS) and cetyltrimethylammonium chloride (CTAC), respectively. The purpose of the study was to identify the relevant factors that control the interactions between the active molecules and their environment in microheterogeneous systems and to evaluate their influence on the protective ability against UV radiation of this family of natural compounds.

Experimental

Extraction and purification of the MAAs

The MAAs, shinorine and porphyra-334, were extracted from the red alga *Porphyra leucosticta* collected from the coast of Mar del Plata city, Argentina, by following the procedure reported by Tsujino *et al.*[26] A 1 : 4 mixture of shinorine and porphyra-334 in water was obtained. The final step in the isolation of the MAAs was achieved by reverse phase HPLC (Konik KNK-500-A) using a Lichrocart® TC-C18 column (5 μm, 4 mm ID × 25 cm), with 0.05% (v/v) aqueous acetic acid as the mobile phase, and UV absorbance detection at 334 nm. Two chromatographic peaks appeared at approximately 4.7 min for shinorine and 8.5 min for porphyra-334. Their assignment was based on an analysis of standard samples previously identified in our laboratory. Each of the fractions was concentrated and stored at −20 °C. The molar absorption coefficients at 334 nm for shinorine (ε = 44 700 M^{-1} cm^{-1})[26] and for porphyra-334 (ε = 42 300 M^{-1} cm^{-1})[27] were respectively used to evaluate the concentration of the solutions.

Preparation of the micellar solutions

SDS (Sigma-Aldrich, >99%) and CTAC (Sigma-Aldrich, 25% solution) were used as ionic surfactants. CTAC was previously purified by evaporation to dryness and recrystallization from a 50% ethanol–acetone mixture. The solid detergent was dissolved in tridistilled water to achieve 0.10 M concentration. An aliquot of a concentrated solution of the MAA in the same solvent was added into the corresponding micellar solution. Unless otherwise indicated, the final pH of the mixtures was around 6.

Determination of K_{ow}

The *n*-octanol/water partition constant, K_{ow}, of the MAAs was evaluated by absorption spectrophotometry. The same volume of *n*-octanol (Sigma-Aldrich, ≥99%) was added to an aqueous solution of a 1.3×10^{-5} M mixture of shinorine and porphyra-334 (1 : 4) at pH 6. The system was stirred and then allowed to equilibrate at 25 °C for three days. The phases were separated by decantation and the absorption spectrum of each of the fractions was recorded. The value of K_{ow} was calculated according to the following expression:

$$K_{ow} = \frac{(A_i - A_f)V_w}{(A_f)V_o} \quad (1)$$

where A_i and A_f refer to the initial and final absorbances at 334 nm of the aqueous fraction, and V_w and V_o are the volumes of the aqueous and organic phases, respectively.[28]

Electrophoresis analysis

The 1 : 4 mixture of MAAs was analyzed by electrophoresis on cellulose paper. The paper strips were alternatively soaked with buffer solutions at pH 2.0 ($H_2PO_4^-/H_3PO_4$, 0.03 M) or pH 5.7 ($HPO_4^{2-}/H_2PO_4^-$, 0.03 M) (Sigma, ≥99%). A voltage of about 20 V was applied between the ends of the strips for approximately 15 min. The location of the MAAs was detected

by staining with 0.5% ninhydrin (UCB p.a., Belgium) in ethanol (Biopack, >96%).

Determination of acidity constants

The apparent pK_a of shinorine and porphyra-334 were experimentally evaluated in aqueous and in micellar solutions of a mixture of these MAAs by potentiometric titration and the Gran method.[29,30] This technique is generally applied to the determination of the pK_a values of amino acids, peptides and small chain fatty acids.[31] In the treatment for V_A ml of a weak acid (HA) with initial concentration C_A and the addition of V_B ml of a strong base (B), the J function is considered to be defined as:

$$J = V_B \times 10^{-pH} = K_a(V_e - V_B) \quad (2)$$

where $V_e = (V_A C_A)/C_B$ denotes the equivalent volume, and K_a is the acid constant of HA. Then K_a can be determined from the slope of the linear regression of the data in a plot of the values of the J function *vs.* V_B.

Mixtures of 4.3×10^{-5} M MAAs in final concentration were alternatively prepared in water or in the presence of 0.1 M SDS or CTAC surfactants. Drops of 1.5 M HCl (Cicarelli, 38%) were added to the MAAs solution before starting the volumetric titration in order to reach an initial pH of about 1.5. The titrating basic solution (0.09 M NaOH Merck, p.a.), was previously standardized against potassium hydrogen phthalate (Fluka, 98%). The ionic strength was kept constant by the addition of solid NaCl (Cicarelli, p.a.) until the final concentration of 0.1 M was reached. The pH values during the titration were acquired at room temperature and under constant stirring by using an HI 2211 pH/ORP Hanna Instruments pH meter equipped with a glass electrode.

A predictive approach based on linear free energy relationships was also applied to approximate the acidity constants of the MAAs. The pK_a were estimated from the chemical structure of each compound and the Hammett equation by the ACD/Lab program.[32]

Light absorption and photostability

The UV-visible absorption spectra of the MAAs in the presence of the SDS or CTAC direct micelles were measured in 1 cm-optical path cells with a double beam UV-visible spectrophotometer (Shimadzu UV-2101 PC). The initial concentration of the MAAs was *ca.* 1.5×10^{-5} M.

The photodecomposition quantum yield of each MAA in the presence of direct micelles was evaluated by steady irradiation in a quartz cell (1 cm-path length) with a polychromatic source (UV lamp CLEO Philips HPA 400S) and an optical filter (cut-on wavelength 320 nm). Reference experiments in the absence of surfactant were carried out with the same MAA concentration and irradiation setup.

The time evolution of the MAA concentration in the irradiated solutions was recorded by UV-visible spectrophotometry. All the experiments were performed in triplicate, at room temperature and with magnetic stirring.

The reported photodecomposition quantum yields in water, Φ_d^w, for shinorine and porphyra-334 (3.4×10^{-4} and 2.4×10^{-4}, respectively)[23] were taken as reference parameters for the calculation of the photodecomposition quantum yields of the same MAA in the micellar media, Φ_d^m, according to eqn (3).

$$\Phi_d^m = \frac{v_d^m}{v_d^w} \cdot \Phi_d^w \quad (3)$$

where v_d indicates the initial photodecomposition rate, and m and w denote micellar medium and water solution, respectively. The photodecomposition rates were obtained from the slope of the linear regression of the plots of the absorbance at 334 nm *vs.* irradiation time.

Emission properties

Fluorescence spectra were measured by means of a Spex Fluoromax spectrofluorometer. The fluorescence quantum yields Φ_f^m of each of the MAA in the micellar solution were determined from the corresponding fluorescence emission spectrum obtained with excitation at 330 nm, by using eqn (4):

$$\Phi_f^m = \Phi_f^w \left(\frac{I_m}{I_w}\right)\left(\frac{A_w}{A_m}\right) \quad (4)$$

where I is the area of the emission band and A is the absorbance at the excitation wavelength.[33] Individual aqueous solutions of shinorine and porphyra-334 were taken as the fluorescence standards with their respective reported fluorescence quantum yields: 1.6×10^{-4} and 2.0×10^{-4}.[23]

The steady-state fluorescence anisotropy $\langle r \rangle$ was determined as described by Hernández *et al.*[34] in a FluoroMax-4 (Horiba Jobin Yvon) instrument with an automated polarization system and a 150 W Xe lamp as the light source. The excitation wavelength was λ_{exc} = 330 nm and the detection was achieved at λ_{em} = 420 nm.

The fluorescence lifetimes were obtained by the time-correlated-single-photon-counting (TCSPC) technique in a Fluorolog-3 TCSPC-SP (Horiba Jobin Yvon) instrument equipped with a nanoLED as the excitation source (λ_{exc} = 340 nm), an iHR320 monochromator, a TBX-04 photomultiplier tube and a FluoroHub-B control module. The signal acquisition was performed at 420 nm by collecting 5000 counts.

Transient absorption experiments

The laser flash photolysis technique was used for the study of the triplet state of MAAs in the presence of the direct micelles. The instrumental setup consisted of a Nd:YAG laser (Spectron Laser SL400) that emits 18 ns pulses, and a 150 W Xe lamp as the source of the analyzer beam. This beam was passed through a water filter to prevent IR radiation impinging on the sample. The detection system included a PTI monochromator coupled to a Hamamatsu R666 photomultiplier. The signals were acquired by a digital oscilloscope (Hewlett-Packard 54504) and transferred to a PC for processing. Averaged signals were recorded after 5 to 15 laser shots.

Benzophenone (Bz) (Aldrich, ≥99%) was used as the triplet sensitizer for the photolysis at 266 nm. Micellar, aqueous and methanol solutions were prepared in the following concentrations: 1×10^{-4} M in porphyra-334 or 4×10^{-5} M in shinorine, and 3×10^{-5} M in Bz. Under these conditions the fraction of Bz triplets deactivated by the MAA is estimated to be between 80% and 90%, if we consider the diffusion limit for the rate of the energy transfer process.[35] All of the photolysis mixtures were previously deaereated by bubbling with argon for 20 minutes.

Results and discussion

The partition coefficient of a compound in the biphasic system *n*-octanol–water, *i.e.* K_{ow}, is considered a good measure of its hydrophobicity. This parameter provides a way of characterizing the partition of the substance between water and nonpolar environments which are common in nature as well as in model systems, such as micellar solutions.[36]

The aqueous mixture of the two structurally related MAAs, shinorine and porphyra-334, showed a small decrease in the absorbance once equilibrated with *n*-octanol at room temperature. From the change in the absorbance at 334 nm of the equilibrated phases, and according to eqn (1), the K_{ow} is estimated to be 0.07 ± 0.01. This value confirms the limited affinity of these MAAs for the organic phase in comparison to water, and suggests a preferential location of the metabolites in the hydrophilic portions of micellar solutions.

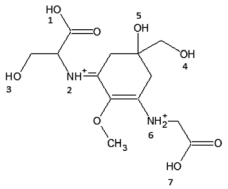
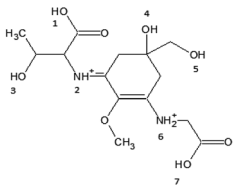
As already mentioned, the acid–base properties of the MAAs are important in describing the species distribution and to rationalize the relevant interactions in a microheterogeneous environment.

An early report by Sivalingam *et al.* affirms that pK_a amounts to 5.7 for the first acid dissociation constant of porphyra-334 in aqueous solution.[37] However, this value is quite far from those corresponding to the free amino acids glycine (pK_a = 2.34 and 9.58) and threonine (pK_a = 2.20 and 8.96),[38] both structurally related to the substituents in the cyclohexenimine core of this MAA.

An electrophoresis analysis performed on the mixture of shinorine and porphyra-334 indicated no appreciable displacement of the MAAs at pH 5.7, and their migration towards the negative pole at pH 2.0. This demonstrates that the compounds are positively charged at the lowest pH, but electrically neutral at pH 5.7, as could be expected for amino acid moieties in the zwitterionic form.

Although the experiments were carried out on solutions containing both shinorine and porphyra-334, the minor structural difference between the two molecules is not expected to significantly affect their tendency to acid dissociation. Furthermore, the results in Table 1 for the acidity constants estimated for the fully protonated species of porphyra-334, based on Hammett's linear correlation, are very similar to those for shinorine, although none of them matches the pK_a value reported by Sivalingam *et al.*[37]

Table 1 pK_a values for the MAAs as predicted from Hammett's correlation

MAA	Molecular structure	Predicted pK_a
Shinorine		1. 1.05 ± 0.40 2. 4.09 ± 0.70 3. 15.09 ± 0.10 4. 14.76 ± 0.10 5. 12.98 ± 0.60 6. 7.12 ± 0.40 7. 1.91 ± 0.10
Porphyra-334		1. 2.16 ± 0.10 2. 4.69 ± 0.70 3. 15.16 ± 0.20 4. 14.77 ± 0.10 5. 13.02 ± 0.60 6. 7.22 ± 0.40 7. 1.14 ± 0.10

In addition, the incorporation or adsorption of the molecules to micelles may change their acid–base properties since both the effective dielectric constant of the solubilization site and the charge distribution of the counterions in the double layer may influence the pK_a.[39] In order to assess these effects, the apparent pK_a for the mixture of MAAs was evaluated in water and in the presence of the charged micelles (SDS or CTAC) by a potentiometric method. Fig. 1 shows the potentiometric curves obtained for the titration with 0.09 M NaOH. Only one plateau corresponding to the lowest pK_a of the compound can be distinguished at pH < 2. The value of the acid constant was derived from each curve by the Gran method by taking the slope of the plot of the *J* function *vs.* V_{NaOH} (see eqn (2)). The inset of Fig. 1 exemplifies this graph for the titration in the presence of CTAC micelles. The results for all the explored media are summarized in Table 2.

The value of pK_a in aqueous solution is within the range of the predictions by Hammett's equation for the carboxylic

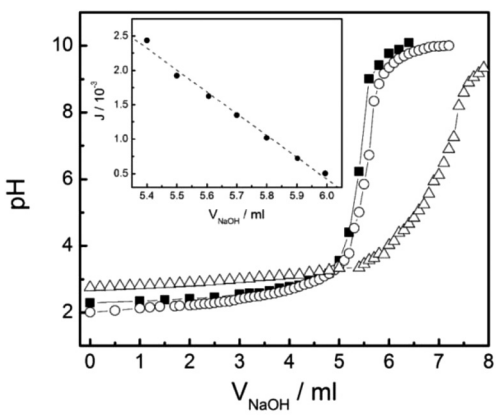


Fig. 1 Potentiometric curve for the titration of MAAs (4.3×10^{-5} M) with NaOH (0.09 M) in: H_2O (-■-), 0.1 M SDS (-○-), and 0.1 M CTAC (-Δ-). Inset: Plot of *J vs.* V_{NaOH} for the titration of the MAAs in 0.1 M CTAC. The dotted line represents the linear fit of the data.

Table 2 Apparent pK_a values for the mixture of MAAs, shinorine and porphyra-334, determined by the Gran method in different environments

Medium	pK_a
H_2O	2.19 ± 0.11
SDS	2.20 ± 0.10
CTAC	2.50 ± 0.10

groups in the two MAAs (see Table 1). In addition, it is consistent with the reported pK_a assigned to this functional group in the free amino acids serine, threonine and glycine (2.1 to 2.4),[38] *i.e.* those related to the substituent pattern in the chemical structures of porphyra-334 and shinorine.

The information in Table 2 shows that the micellar effect on the acid dissociation of the carboxylic functional group is slight. The largest difference is verified in the case of the micelles formed by the cationic surfactant CTAC, where the pK_a is raised by *ca.* 0.3 units compared to water. This effect can be explained by the proximity of the MAA to the micelle surface. The lower dielectric constant of the micelle–water interface favors the uncharged species, thereby inhibiting the deprotonation of the carboxylic acid groups. The magnitude of the observed ΔpK_a is also in line with the dielectric effect, since the charge effect is expected to be much larger for ionic micelles and even opposite in the case of the cationic ones.[39]

The UV-visible absorption spectra of the individual MAAs porphyra-334 and shinorine were examined in the presence of the micelles as well as in the neat solvent. Fig. 2 illustrates the results for porphyra-334 in: water, 0.1 M SDS and 0.1 M CTAC. In all cases, the single band is located around 334 nm. A similar condition is found for shinorine (data not shown). A summary of the maximum wavelengths for the two MAAs in the different environments can be found in Table 3.

The absorption spectra of the MAAs in the presence of SDS micelles do not change in comparison to those in water solution. But a slight bathochromic shift is observed in the case of CTAC. The small effect suggests that the relative stabilization of the excited state is not significantly modified with respect to

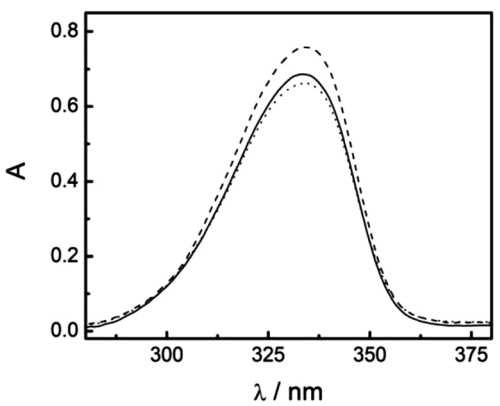


Fig. 2 UV absorption spectra of porphyra-334 in different media: H_2O (···), 0.1 M SDS (——), and 0.1 M CTAC (- - -).

Table 3 Photophysical and photochemical parameters for the MAAs in different media

MAA	Shinorine			Porphyra-334		
Medium	H_2O	SDS	CTAC	H_2O	SDS	CTAC
λ_{abs}/nm	334.0	334.0	334.5	334.0	334.0	334.5
λ_{em}/nm	424.0	424.0	418.0	426.0	426.0	406.0
$\Delta\bar{\nu}$ cm^{-1}	6355	6266	5971	6466	6466	5264
$\Phi_f/10^{-4}$	1.6	1.6	3.3	2.0	1.9	3.4
τ_f/ns	0.58	0.75	0.87	0.22	0.35	0.47
$\langle r \rangle$	0.085	0.086	0.100	0.070	0.116	0.132
$\Phi_d/10^{-4}$	3.4	3.1	1.2	2.4	1.9	1.0

the situation in aqueous solution. This is consistent with the magnitude of the K_{ow} partition constant, according to which the MAAs are expected to preferentially solubilize in the aqueous region outside the micelles. Still, electrostatic interactions with the polar heads of the ionic surfactants are likely to occur in the micellar systems since the MAAs are present in the form of zwitterions at the pH ~ 6 of the solutions.

The fluorescence spectra recorded for each of the MAAs in water and in the micellar solutions are evidence of a clearer trend. Despite the relatively low contribution of the radiative relaxation pathway of the electronic excited states of MAAs,[23,24,35] the emission spectra are noticeably affected in the presence of micelles (Fig. 3).

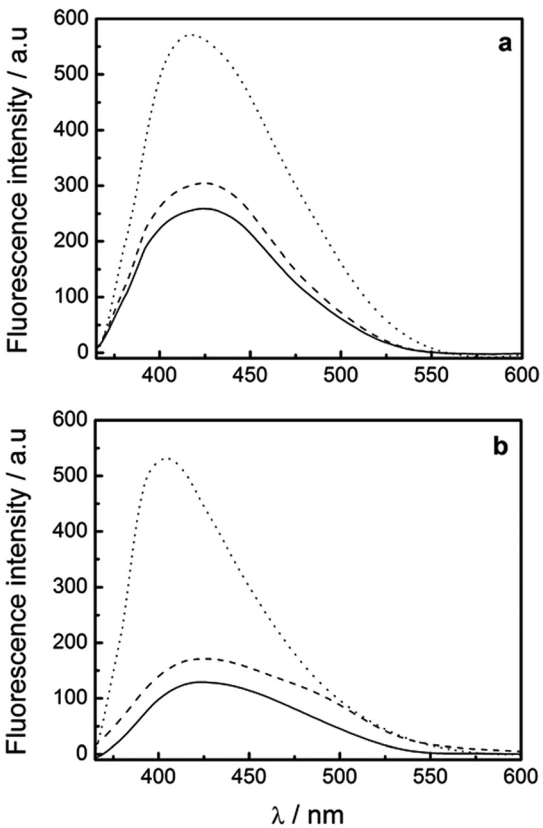


Fig. 3 Fluorescence emission spectra of (a) shinorine and (b) porphyra-334, in different media. H_2O (——), 0.1 M SDS (- - -), and 0.1 M CTAC (···).

Appreciable blue shifts (6 to 20 nm) of the fluorescence maximum and an increase in the intensity of the bands are observed for the CTAC solutions when excited at 330 nm. However, the emission bands in SDS and in water solutions look the same (see Table 3). In addition, the excitation spectra obtained for the emission at 420 nm coincide with the UV absorption of both MAAs (data not shown), corroborating the origin of the emission.

The fluorescence quantum yields were calculated from eqn (4), by considering the reported value for the corresponding MAA in aqueous solution as a reference.[23] The results in Table 3 for both MAAs confirm almost two-fold increments in the emission yields in CTAC, whereas in the presence of SDS the values of Φ_f are comparable to those in aqueous solutions.

Based on the information about the absorption and emission bands, the Stokes shifts ($\Delta\bar{\nu}$) were calculated. The results are also listed in Table 3. Their comparison confirms the distinctive behavior in the presence of CTAC micelles. The values of $\Delta\bar{\nu}$ are between 300 and 1200 cm^{-1} lower than those in water and SDS solutions. Stokes shifts usually correlate with the orientation polarizability of the molecules. Thus, the results suggest that the decrease in $\Delta\bar{\nu}$ is due to the relatively strong electrostatic attractions with the CTAC micellar surface and the consequent limitation of the solute movement in that environment.

The mobility of a fluorophore can also be assessed through the steady-state anisotropy <*r*>. This parameter was evaluated for both MAAs in the micellar and homogeneous media. The results in Table 3 show rather increased values of <*r*> for the direct micelles in comparison with aqueous solution. The differences are consistent with the occurrence of interactions between the MAAs and the surfactants that restrict their diffusive motion.[40] The increments in the case of porphyra-334 are more significant than those for shinorine, which may be explained by the extra methyl group in the chemical structure of the former exerting some degree of hindrance in the reorientation movements. Moreover, the effect on <*r*> in the presence of CTAC is more important than in SDS. This is consistent with the larger interactions with the cationic surfactant suggested by the rest of the fluorescence parameters presented in Table 3. Similar trends have been reported for the flavonoid quercetin in the same direct micelles; the changes were ascribed to the electrostatic interactions of the molecules with charged microheterogeneous environments.[41]

Time-resolved studies of the fluorescent deactivation were carried out by TCSPC on individual solutions of shinorine and porphyra 5×10^{-6} M. Biexponential functions were used to adjust the decays, with residues limited to the range of ±4, so the fit was considered satisfactory. In all cases, an exponential contribution amounting *ca*. 95% of the signal was assigned to the fluorescence lifetime (τ_f). The values of τ_f, summarized in Table 3, show a slight tendency to increase in the presence of CTAC micelles. Restriction of the fluorophore movements may also explain the origin of this effect, which in turn decreases the efficiency of the non-radiative decay. The thermal deactivation is the predominant relaxation pathway of photon activated MAAs. This is a very fast process in which changes occur in the molecular geometry through funnels (conical intersections) of the potential energy surfaces, while the excitation energy is delivered as heat to the environment.[25] The mechanism explains the rapid population of the lowest electronic state and correlates with the direct experimental evidence from fluorescence and laser-induced optoacoustic spectroscopies that provide kinetic information on the excited state relaxation pathways.[23,24]

The photostability of the MAAs in the presence of micelles was evaluated in terms of the photodecomposition quantum yields (Φ_d). The initial rates of the photoinduced degradation in SDS, CTAC and aqueous solutions were derived from the decays of the absorbance at 334 nm with the irradiation time (Fig. 4). A linear behavior is observed for all the systems. The yields were determined from the slope of the linear regression of these plots and eqn (3). The results are summarized in Table 3.

It can be observed that the extent of the photolysis in microheterogeneous systems is comparable with the results reported by Conde *et al.* for the natural compounds in aqueous solu-

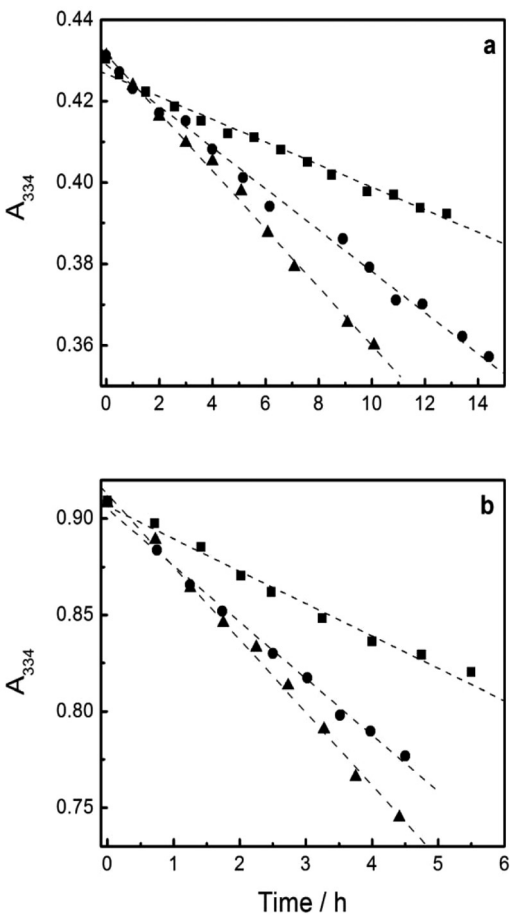


Fig. 4 Plots of the absorbance at 334 nm for: (a) shinorine and (b) porphyra-334 *vs.* irradiation time, in different media: H_2O (-▲-), 0.1 M SDS (-●-), and 0.1 M CTAC (-■-).

tion.[23] The Φ_d's are lower than 0.04% in all these media, confirming the high photochemical stability of the molecules. However, a comparative analysis of the values in Table 3 indicates between a 2.4- and a 3-fold decrease for porphyra-334 and shinorine, respectively, in the presence of CTAC micelles with respect to water. The yields also drop in the SDS solutions, but by less than 20%. Coincidently with the trends observed in the photophysical properties for the different media, the photochemistry of MAAs exhibits a greater effect in the cationic micelles CTAC which cause, in this case, an extra stabilization. While the origin of this protection against photodegradation is not clear, the effect could also be related to the local microviscosity in the water/micelle interface, adding some degree of hindrance to the molecular movements that promote the passage to the reactive channels in the potential energy surface of the excited state.

In summary, these results point to noticeable interactions in the presence of CTAC which are not operative for SDS. Due to the cationic nature of CTAC, it is proposed that the carboxylate groups of the MAAs are responsible for the attractions with the positively charged heads of the detergent. In the case of the anionic SDS micelles, these groups are repelled so that the MAAs are more likely to reside in the aqueous environment, as suggested by the similar magnitudes of the properties measured in these two media. This implies that the positive charges of the amino groups in the zwitterionic MAAs may not be involved in the coulombic interactions with ionic detergent surfaces. Furthermore, the optimized geometries arising from molecular calculations for these MAAs or related structures are consistent with spatial arrangements of the N atoms with minor exposure to the molecule surface.[25,42]

Still, several of the parameters determined here for the SDS solutions differ, although to a reduced extent, from those in water. This could be attributed to a weaker interaction such as that arising from ion pairing with SDS monomers in the aqueous phase.

Finally, the absorption spectra of the MAAs in an excited triplet state were assessed in the micellar media. The triplet states were generated by photosensitization with Bz at 266 nm, since the low intersystem crossing yields of MAAs prevent them being obtained from direct photolysis.[23] The spectrum of triplet Bz was also obtained under the same conditions but in the absence of MAAs, *i.e.* two bands centered at 330 nm and 550 nm (data not shown), which coincide with the maxima reported in the literature for this species.[43]

Fig. 5 shows the transient spectra for each MAA in the presence of Bz obtained 10 microseconds after the laser pulse. Broad bands in the zone of 350–400 nm were observed for both MAAs. They are assigned to triplet–triplet transitions.[23,35] In the presence of the direct micelles the maximum of the absorption due to triplet porphyra-334 shows a hypsochromic shift with respect to the spectrum in aqueous solution (Fig. 5b). For SDS micelles the behavior is similar to that observed in aqueous solution for both porphyra-334 and shinorine. The absorption is less intense, however, in CTAC, and the spectrum resembles that registered in methanol.

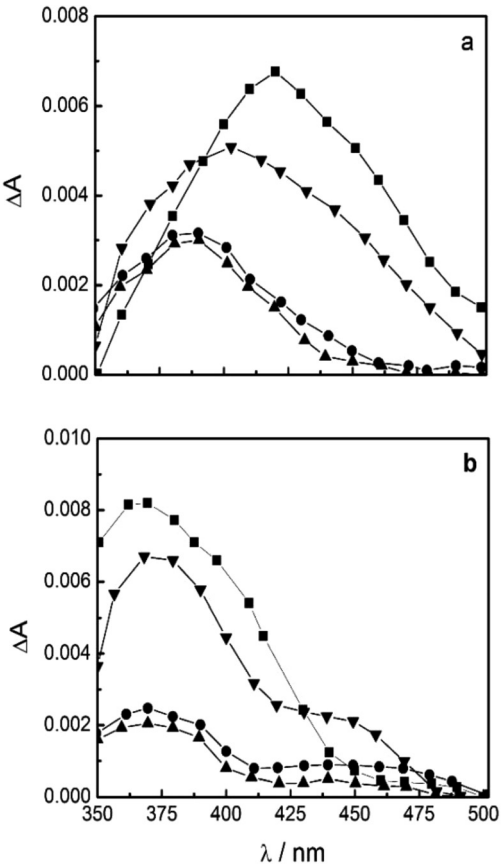


Fig. 5 Benzophenone photosensitized triplet–triplet absorption spectra obtained 10 μs after the 266 nm laser pulses for: (a) shinorine and (b) porphyra-334 in: H_2O (■), CH_3OH (●), 0.1 M SDS (▼), and 0.1 M CTAC (▲).

This similarity may be explained by considering the polarities of the interface of these organized systems, which are comparable to that of *n*-alcohols.[44] This in turn supports the conclusion about MAAs locating close to the surface of the cationic micelles.

Conclusions

The hydrophobicity and the effects on the acid–base and photophysicochemical behavior of the MAAs shinorine and porphyra-334 in the presence of charged direct micelles were evaluated.

According to the low value of K_{ow}, the preferential location of the MAAs is the aqueous portion of the micellar systems.

The acid–base equilibrium related to the carboxylic groups of the natural molecules is negligibly affected by anionic SDS micelles, but the cationic surfactant CTAC slightly inhibits their tendency to proton dissociation. Thus, in all these media and near the physiological pH, the MAAs are expected to be in the form of zwitterions.

Under these conditions, the micellar effects are better manifested in the emission process, mainly in CTAC where the

fluorescence quantum yield is enhanced and the lifetime increases. These results, together with the observed Stokes shifts and stationary anisotropies, suggest hindrance of the reorientation molecular movements of the MAAs due to electrostatic attractions between the carboxylate groups and the cationic heads of CTAC on the surface of the micelles.

Consistent with this, the absorption spectrum associated with triplet–triplet transitions in water is similar to those determined in the presence of SDS micelles, while in CTAC the spectral features resemble those observed in methanol, a solvent considered to emulate the polarity of micellar interfaces.

In addition, the photostability of the MAAs improves in the micellar systems. The effect is also more noticeable in the case of CTAC and is probably related to the hindrance of molecular movements in the micelle/water interface that are involved in access to the reactive channels of the excited states.

In summary, the properties determined in this work confirm that one of the most relevant roles attributed to the MAAs, which is related to their ability to act as efficient biological UV screens, is preserved in organized microheterogeneous environments such as ionic micelles. Moreover, the chemical reactivity slows down in the presence of CTAC along with an increase in the fluorescence yield. However, since a clue to the UV photoprotective function of MAAs is the highly efficient nonradiative deactivation of their excited states, one should take into account that the competition of the radiative pathway may compromise the efficiency of protection by enabling trivial mechanisms of energy transfer from fluorescence.

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