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Absorption and emission spectroscopic investigation of alloxazine in aqueous solutions and comparison with lumichrome

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ABSTRACT

The absorption and emission spectroscopic behavior of alloxazine is studied in aqueous solutions at pH 4 (10^{-4} M HCl) and pH 10 (10^{-4} M NaOH). At pH 4 only the pure form of alloxazine is present, while at pH 10 about 9% of alloxazine is present in its tautomeric form of isoalloxazine. Absorption cross-section spectra, fluorescence emission quantum distributions, fluorescence excitation quantum distributions, excitation wavelength dependent fluorescence quantum yields, fluorescence lifetimes, and radiative lifetimes are determined. The spectroscopic behavior of alloxazine at pH 4 and pH 10 is compared with that of 7,8-dimethyl-alloxazine (lumichrome) at pH 4 and pH 10. Photo-induced (excited-state) tautomerization of alloxazine to isoalloxazine is observed which does not occur for lumichrome. In the case of alloxazine the highest occupied molecular n orbital (n-HOMO) is energetically well below the highest occupied molecular π orbital (π-HOMO) giving non-degenerate ground-state to first excited-state (ππ*) transition. For isoalloxazine, lumichrome, and lumichrome-10H-tautomer n-HOMO is only slightly below π-HOMO causing photo-induced ground-state n → π electron transfer with elongation of fluorescence lifetime and radiative lifetime.

1. Introduction

Alloxazine and its tautomeric form isoalloxazine (see structural formulae in Fig. 1) are the structural parents of the broad family of flavins of which prominent members are lumichrome (7,8-dimethyl-alloxazine), lumiflavin (7,8,10-trimethyl-isoalloxazine), riboflavin (7,8-dimethyl-10-ribityl-isoalloxazine, vitamin B_2), flavin mononucleotide (7,8-dimethyl-10-ribityl-dihydrogenphosphate-isoalloxazine, FMN) and flavin adenine dinucleotide (7,8-dimethyl-10-ribityl-diphosphate-adenosine-isoalloxazine, FAD) [1–3].

Previous investigations of alloxazine in aqueous solutions are scarce [4–7]. Spectroscopic and photo-physical studies on alloxazine in various other liquid and solid solvents are found in [4–11]. But investigation of alloxazine in aqueous solution is most important because of its biological relevance. Instead of alloxazine in aqueous solution its derivative lumichrome (7,8-dimethyl-alloxazine) in aqueous solution has been extensively studied [12–18]. Lumichrome is the main photo-degradation product of flavins in aqueous solutions below pH 7 [1,19–25]. The solubility of lumichrome in water [26,27] (solubility limit concentration C_{sol} = 19 μM in distilled water at 25 °C [26]) is slightly higher than that of alloxazine in water [28,29] (C_{sol} = 9.05 ± 1 μM at pH 4 and 21 °C [29], C_{sol} = 14.5 ± 1 μM at pH 10 and 21 °C [29], C_{sol} = 15.5 μM in double distilled water at 25 °C [28]).

Here the absorption and emission spectroscopic behavior of alloxazine in aqueous solutions at pH 4 and pH 10 is studied and quantitative analyzed including the determination of the efficiency of photo-induced alloxazine to isoalloxazine tautomerization and the determination of the energy difference between the highest occupied molecular π orbital (π-HOMO) and the highest occupied molecular n orbital (n-HOMO) for alloxazine and isoalloxazine. The obtained results for alloxazine in aqueous solution at pH 4 and pH 10 are compared with those of lumichrome in aqueous solution at pH 4 and pH 10. The lumichrome data are taken from [16]. Quite surprising differences between alloxazine and lumichrome are revealed (absorption strength, fluorescence lifetime, fluorescence quantum yield, thermal ground-state tautomeric content at pH 10, photo-tautomerization of alloxazine but no photo-tautomerization of lumichrome, photo-induced ground-state n-π electron transfer of lumichrome, lumichrome-10H-tautomer, and isoalloxazine, no photo-induced ground-state n-π energy transfer for alloxazine).

2. Experimental

Alloxazine was purchased from Aldrich (purity 96%) and used as delivered. The pH 4 aqueous solvent was prepared by diluting 1 M

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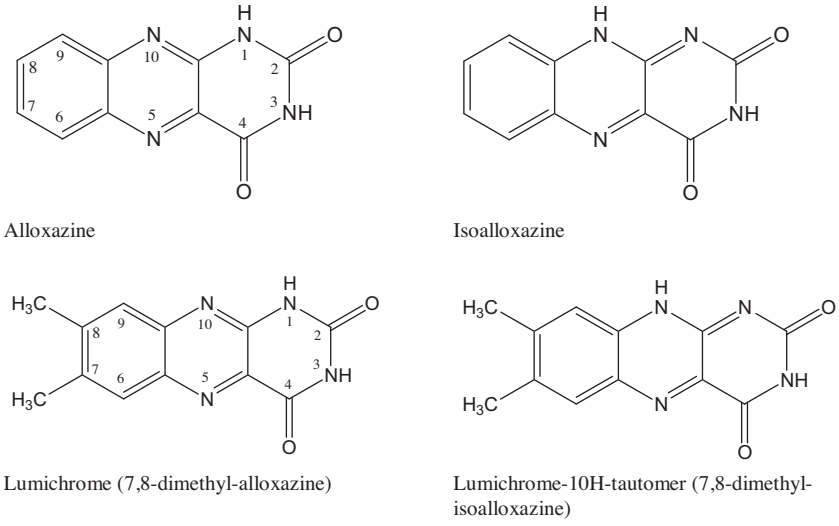

Fig. 1. Structural formulae of alloxazine (A), isoalloxazine (IA), lumichrome (LC) and lumichrome-10H-tautomer (LCH). Molar mass of A and IA: 214.18 g mol^{-1}. Molar mass of LC and LCH: 242.23 g mol^{-1}.

HCl from Fluka to 1×10^{-4} M HCl with Millipore water. The pH 10 aqueous solvent was prepared by diluting 1 M NaOH from Merck to 1×10^{-4} M NaOH with Millipore water.

Absorption coefficient spectra measurements $\alpha_a(\lambda)$ were carried out with a commercial spectrophotometer (Cary 50 from Varian). The absorption cross-section spectra $\sigma_a(\lambda)$ were calculated from the absorption coefficient spectra by the relation $\sigma_a(\lambda) = \alpha_a(\lambda)/N_0$ where N_0 is the alloxazine molecule number density.

Fluorescence emission spectra (fluorescence excitation wavelength $\lambda_{F,exc}$ fixed) and fluorescence excitation spectra (fluorescence detection wavelength $\lambda_{F,det}$ fixed) were recorded with a commercial fluorimeter (Cary Eclipse from Varian) under magic angle polarization conditions (vertical polarized excitation, polarizer in fluorescence detection path oriented under an angle of 54.7° to the vertical) [30]. The spectra were corrected for spectral sensitivities of the spectrometers and the photo-detector (emission and excitation spectra), for fluorescence re-absorption (emission and excitation spectra) and for excitation light depletion (excitation spectra) [31–33]. For absolute intrinsic fluorescence quantum distribution $E_F(\lambda)$ [34] and fluorescence quantum yield ϕ_F determinations the dye POPOP (1,4-di-(5-phenyloxazolyl) benzene) in ethanol (fluorescence quantum yield $\phi_F = 0.85$ [35]) was used as reference standard.

Fluorescence lifetime measurements were performed under magic angle polarization conditions with second harmonic picosecond laser excitation pulses (wavelength 400 nm, duration 3 ps) of a mode-locked titanium sapphire oscillator—regenerative amplifier laser system (Hurricane from Spectra-Physics) and real-time fluorescence signal detection with a micro-channel-plate photomultiplier (Hamamatsu type R1564U-01) connected to a fast digital oscilloscope (LeCroy type 9362) [36]. The fluorescence emission in near-backward direction was collected for the temporal fluorescence trace measurements. The laser system was operated in single shot mode. Each fluorescence signal curve presented below is an average over ten single traces. The time interval between successive recordings (laser shots) was about 2 min.

3. Results and discussion

The absorption and emission behavior of alloxazine was studied in aqueous solution at pH 4 and pH 10. Its behavior is compared with lumichrome also in aqueous solution at pH 4 and pH 10. Extracted parameters for alloxazine and lumichrome are collected in Table 1. The selection of aqueous solvents at pH 4 and pH 10 followed the experience on pH dependent spectroscopic investigations of lumichrome [16]: At pH 4 and pH 10 alloxazine is expected to be present only in neutral form. At pH 4 alloxazine is expected to be free of a tautomeric isoalloxazine contribution, while at pH 10 a partial presence of isoalloxazine is expected.

3.1. Absorption behavior

Determined absorption cross-section spectra of alloxazine in 10^{-4} M HCl (pH 4) and 10^{-4} M NaOH (pH 10) are depicted in the top part of Fig. 2. The applied alloxazine concentrations of $C = 2.76 \times 10^{-6}$ mol dm^{-3} in pH 4 solution and $C = 5.01 \times 10^{-6}$ mol dm^{-3} in pH 10 solution are well below the solubility limit concentrations [29].

At pH 4 the absorption spectrum of pure alloxazine is observed (solid curve in top part of Fig. 2 with steep long-wavelength absorption decrease). The absorption spectrum of alloxazine in pH 10 solution (dashed curve in top part of Fig. 2) exhibits a weak long-wavelength absorption extension out to about 480 nm indicating the presence of part of alloxazine in its tautomeric form of isoalloxazine (for structural formulae see Fig. 1). The absorption cross-section curve $\sigma_a(\lambda)$ of alloxazine in aqueous solution at pH 10 is given by

$$\begin{aligned}\sigma_a(\lambda) &= (1 - x_{S_0,IA})\sigma_{a,A}(\lambda) + x_{S_0,IA}\sigma_{a,IA}(\lambda) \\ &= \sigma_{a,A}(\lambda) + x_{S_0,IA}[\sigma_{a,IA}(\lambda) - \sigma_{a,A}(\lambda)]\end{aligned} \quad (1a)$$

where $\sigma_{a,A}(\lambda)$ is the absorption cross-section spectrum of unchanged alloxazine (absorption spectrum of alloxazine at pH 4), $\sigma_{a,IA}(\lambda)$ is the absorption cross-section spectrum of isoalloxazine (alloxazine-tautomer), and $x_{S_0,IA}$ is the mole-fraction of isoalloxazine in the S_0 ground-state. Solving Eq. (1a) for $\sigma_{a,IA}(\lambda)$ gives

$$\sigma_{a,IA}(\lambda) = \frac{\sigma_a(\lambda) - \sigma_{a,A}(\lambda)}{x_{S_0,IA}} + \sigma_{a,A}(\lambda). \quad (1b)$$

The absorption cross-section difference $\sigma_a(\lambda) - \sigma_{a,A}(\lambda)$ (dashed curve–solid curve of top part of Fig. 2) is displayed by the dashed curve in the middle part of Fig. 2. The displayed $\sigma_{a,IA}(\lambda)$ curve (solid curve in middle part of Fig. 2) was obtained for $x_{S_0,IA} = 0.09$.