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Influence of pH on spectral and photophysical properties of 9-methyl-5-deazaalloxazine and 10-ethyl-5-deaza-isoalloxazine

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ABSTRACT

Absorption, emission, excitation and synchronous fluorescence spectra were used to describe protonation/deprotonation equilibria for a derivative of deazaalloxazine, 9-methyl-5-deazaalloxazine (9Me-5-DAll), and its flavin derivative 10-ethyl-5-deaza-isoalloxazine (10Et-5-DIAll). In addition, spectral and photophysical properties of 9Me-5-DAll and of 10Et-5-DIAll were compared to those of methyl derivatives of alloxazine, such as lumichrome or lumiflavin (10-methyl-isoalloxazine).

One species only is present in acidic and neutral solutions of 9Me-5-DAll, which absorbs and emits light, in contrast to basic solutions where three such species are present, identified as the two monoanions and one dianion. The analysis of steady-state and time-resolved spectra predict that the N(3) monoanion of 9Me-5-DAll has "alloxazinic" structure while its N(1) monoanion and N(1,3) dianion have predominantly isoalloxazinic structures. The lifetimes of these forms were determined at selected pH values. Similarly, for 10Et-5-DIAll there is one protonated species at low pH values. At higher pH, this species undergoes deprotonation forming the neutral molecule, which is present in aqueous solutions up to pH 12. At pH > 12 the monoanionic, isoalloxazinic form of 10Et-5-DIAll is predominant. Based on the analysis of the time-resolved spectra of the latter species, we conclude that its protonated form has no detectable fluorescence. Additionally, time-resolved spectra indicate that at pH > 11 there are two emitting species in solution. The first is identified as the isoalloxazinic monoanion of 10Et-5-DIAll, and the second as the tautomeric form of this monoanion, which is formed in the excited state.

Based on the measurements, we also estimated pK_a values of the two compounds for the equilibria between the individual species both in the ground and excited states.

1. Introduction

Alloxazines, the main products of photodecomposition of the biologically important flavins, are the group of nitrogen-containing heterocycles. They are present in many foods and formed in the normal metabolic handling of ingested riboflavin [1]. Photophysical and photochemical properties of alloxazines are interesting due to their possible involvement in a wide variety of biological systems [1–3]. The best-known representative of alloxazines is lumichrome (7,8-dimethylalloxazine). The photophysics and photochemistry of alloxazine and its derivatives have been studied since 1966, when proton transfer reactions in lumichrome and related compounds were first discovered [4]. Alloxazines unsubstituted at the N(1) position can undergo excited-state proton transfer from N(1) to N(10). This reaction occurs in the presence of compounds with donor–acceptor properties such as carboxylic acids, e.g. acetic acid [5–10].

The driving force for this process seems to be the change in the electron density on the nitrogen atoms of the molecule upon excitation. Such changes can be estimated by studying the acid–base equilibria of the molecules in the ground and excited state. Spectral and photophysical properties of different derivatives of alloxazines both in solutions and in solid state are the subject of intensive studies in our group [6,10–17].

The most intensive studies on acid–base equilibria were conducted for lumichrome (7,8-dimethylalloxazine) [8,18,19]. For instance, Penzkofer and Tyagi [19] demonstrated that various species are present in lumichrome solutions in the 1–14 pH range, namely its cationic, neutral and monoanionic forms. Additionally, they suggested that above pH 7 a partial ground-state tautomerization of lumichrome to 7,8-dimethylisoalloxazine occurs by intra-molecular proton transfer between the N(1) and N(10) nitrogen atoms. Recently, we also studied acid–base equilibria of lumichrome and its methyl derivatives using steady-state and time-resolved fluorescence [20], experimental and theoretical approaches. Our conclusions are similar to those of Lasser and Feitelson [18], and Koziołowa [8], being in contradiction to those reported by Tyagi and Penzkofer.

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We find that the neutral form of lumichrome is present in the range from pH 1 to pH ≈8. In the range from pH 9 to pH 12 we detect the presence of two monoanions of lumichrome, one at N(1), with alloxazinic structure, and the second at N(3), with isoalloxazinic structure. Our conclusions were confirmed by the fact that 1-methyllumichrome monoanion (with the negative charge located situated at the N(3) atom) has an alloxazinic structure; with typical absorption and emission spectra characteristic for such compounds [16,21,22]. Contrary to that, the 3-methyllumichrome monoanion (with the negative charge located at the N(10) atom) has the isoalloxazinic structure characterized by the isoalloxazinic-type absorption and emission spectra [2]. Our conclusions were also confirmed by the TD-DFT calculations. We also find that the isoalloxazinic dianion of lumichrome becomes predominant at pH > 12. This dianion has isoalloxazinic structure, with negative charges at both the N(3) and N(10) atoms [20].

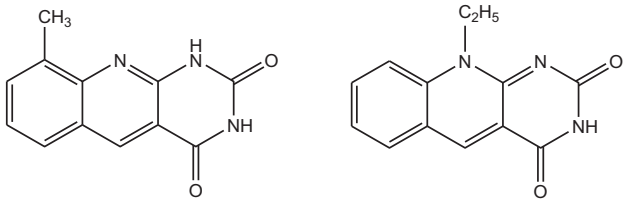
The calculated pK_a for the equilibrium between neutral and anionic forms of lumichrome was 8.2; note that the N(1)-H and N(3)-H groups deprotonate with similar probabilities. The pK_a equals to 11.4 for the equilibrium between the monoanionic and dianionic forms, the latter with charges located at both N(3) and N(10). We also estimated the pK_a* values for the same equilibria, obtaining 7.7 and 11.2, respectively [20].

5-Deazaalloxazines are homologues of 5-deaza-flavins (5-deaza-isoalloxazines), compounds that are cofactors in yellow chromophores [23] and are known as blue-light receptors [24]. 5-Deaza-flavins are potential riboflavin antagonists with their own redox system, different from that of riboflavin [25]. The most important structural difference between alloxazines and 5-deazaalloxazines and between 5-deaza-flavin and flavin is the replacement of the nitrogen atom at position 5 in the molecule by a methine group. These two classes of compounds possess relatively similar structures, with certain similarity in their spectral, photophysical and photochemical properties [25,26]. However, there are very few published data on photophysical properties of 5-deazaalloxazines in contrast to similar alloxazine derivatives. In early works some authors [25] who studied 5-deaza-flavins in comparison to flavins concluded that the chemistry of these compounds differs significantly, thus, 5-deaza-flavin should be considered a "flavin-shaped nicotinamide" rather than a "flavin analogue".

But in spite of this, 5-deaza-flavins were often used as references to establish reaction mechanisms of some flavins in the ground and excited states [27,28].

The acid–base equilibria of 5-deazalumichrome (7,8-dimethyl-5-deazaalloxazine) were studied by Koziołowa. The apparent pK_a for the formation of monoanion, resulting from deprotonation of both N(1)-H and N(3)-H groups of 5-deazalumichrome, is 8.8 in the ground state. However, Koziołowa reported that pK_a* for the formation of the N(1) monoanion in the first excited state is 4.5 and that for the formation of the N(3) monoanion is the same as in the ground state (pK_a* = 8.8). The next step of deprotonation of 5-deazalumichrome creates a dianion with pK_a > 12 [8]. However, we later concluded, on the basis of discussion presented below and our recent work [20], that pK_a values in the ground and in the excited state are similar for this derivative of 5-deazaalloxazine.

Presently we report spectral and photophysical properties at different pH for 9-methyl-5-deazaalloxazine (9Me-5-DAll) and 10-ethyl-5-deaza-isoalloxazine (10Et-5-DIAll), comparing these to the data available for alloxazines, especially for lumichrome [20] and lumiflavin [2,28,29]. We use absorption, fluorescence and fluorescence excitation spectra, together with fluorescence lifetime measurements and some additional spectral methods – synchronous fluorescence spectroscopy – as the technique used to better distinguish the different fluorescent species (i.e. protonated and neutral forms, monoanions, and dianions) present in aqueous solution of the investigated compounds at different pH.



9-methyl-5-deazaalloxazine (9Me-5-DAll) 10-ethyl-5-deaza-isoalloxazine (10Et-5-DIAll)

Fig. 1. Structures of 9-methyl-5-deazaalloxazine (9-Me-5-DAll) and 10-ethyl-5-deaza-isoalloxazine (10Et-5-DIAll).

Fig. 1 presents the structures of target compounds.

2. Experimental

2.1. Materials

Hydrochloric acid and sodium hydroxide were from Aldrich. Water was triply distilled. 9-Methyl-5-deazaalloxazine and 10-ethyl-5-deaza-isoalloxazine were synthesized according to the procedure proposed by Yoneda et al. [30,31] in a two-step synthesis. First (step 1) we obtained the respective methyl-substituted derivatives of 6-anilinouracils, next (step 2) we performed their cyclization using the Vilsmeier procedure.

2.2. Solutions and spectra

At first we prepared solutions of 9Me-5-DAll and 10-ethyl-5-deaza-isoalloxazine, dissolving these compounds in 2 M HCl. Next, an appropriate volume of 2 M NaOH was added resulting at pH ≈ 0. These solutions were titrated with 2 M or 4 M NaOH to the appropriate pH values. The exact pH of each solution was determined using a Hanna Instruments pH-meter. We maintained the same concentration of the titrated compounds in each solution.

All solutions were prepared on the same day as their absorbance, steady-state fluorescence, fluorescence excitation, and synchronous fluorescence spectra were recorded. Also the time-resolved fluorescence measurements were performed at the same time, in order to minimize any photolytic or hydrolysis reactions.

Absorption (using the option to measure absorption), emission, excitation and synchronous fluorescence spectra were recorded on Horiba Jobin-Yvon Spex Fluorolog 3 spectrofluorometer.

The synchronous fluorescence spectra can be described expressing the synchronous fluorescence intensity I_s as a function of the analyte concentration c: [32]

$$I_s = Kcb\,\mathrm{Ex}(\lambda_{ex})\,\mathrm{Em}(\lambda_{ex} + \Delta\lambda) \quad (1)$$

Here, I_s is the synchronous fluorescence intensity; c – the concentration of the analyte, Ex – the intensity of the excitation spectrum at λ_{ex}, Em – the intensity of the emission spectrum at $\lambda_{ex} + \Delta\lambda$, b the thickness of the sample; and K the constant describing the instrumental factors, including geometry and other parameters. The synchronous spectra were recorded in the range from 350 to 500 nm with different offsets ($\Delta\lambda$), although the analysis was performed at $\Delta\lambda = 20$ nm, due to higher sensitivity and better spectral resolution. However, there is no general rule for choosing $\Delta\lambda$. A simple rule is to use small values, 10 or 20 nm (the slit widths should not exceed half of $\Delta\lambda$) for compounds with small Stokes shift, and larger values for compounds with larger Stokes shift. The value of $\Delta\lambda$ has a significant influence on the nature and shape of the spectrum recorded. For example, the synchronous bandwidth increases with increasing $\Delta\lambda$. Normally, a single band is observed in the synchronous scan spectrum for each of the emitting