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Investigation of protonation effects on the electronic and structural properties of halogenated sulfonated porphyrins

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HIGHLIGHTS

- The presence of halogen atoms reduces the flexibility of the porphyrinic ring.
- The protonation was observed only in extremely acid environment.
- Chlorinated atoms cause a stronger effect on the fluorescence than fluorinated atoms.
- Protonation reduces the fluorescence lifetime of sulfonated porphyrins.
- Protonation causes an enhancement of the absorbance in the therapeutic window.

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ABSTRACT

The present study aimed at evaluating pH effects on the electronic and structural properties of halogenated sulfonated porphyrins. We used UV–Vis absorption, fluorescence emission, and NMR spectroscopies, combined with quantum chemical calculations using Density Functional Theory. We observed that the presence of one or two halogen atoms (chlorine or fluorine) at *ortho*-position of phenyl ring reduces the flexibility of the porphyrin structures, making their protonation harder. Since the protonation of anionic porphyrins provides a convenient way for their aggregation, the protonation observed only in extremely acid environment is indicative of the low probability for the aggregation of these porphyrins, which is an important characteristic of an efficient photosensitizer. Furthermore, the enhancement in the absorbance (9–13.5 times) in the phototherapeutic window suggests that the protonation of halogenated sulfonated porphyrins could be a possible strategy for a higher photodynamic efficiency.

Introduction

Porphyrins are an important class of organic molecules which has aroused considerable research interest in the last decades. Investigations on how the external factors or structural modifications influence the electronic properties of porphyrins are a necessary framework for further achievements in many areas like nonlinear optics, photomedicine, light-harvesting systems, molecular wires for electron transfer, molecular switches, chemical sensors and others [1–6]. The most remarkable features of these compounds are: strong absorption in the visible region, high triplet state formation and singlet oxygen generation quantum yield [2]. These are pivotal characteristics for their application as photosensitizers in medicine and pharmacology, e.g., photodynamic action against fungi, bacteria and cancer [2,7,8].

Recently, many efforts have been devoted to synthesize new compounds as well as to investigate their optical and photophysical properties, aiming at finding efficient photosensitizers with improved optical and pharmacokinetic features [9–13]. With this purpose, porphyrin-type molecules (porphyrins, chlorins and bacteriochlorins) with halogen atoms in their structure have been synthesized [12–14], presenting photosensitizing efficacy.

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The main functions and mechanisms of action for porphyrin-type molecules are based on their photophysical characteristics, which are governed by their physicochemical properties. It is well established that modifications in the porphyrin environment or in its molecular structure [15–18] can change its optical and photophysical features. For instance, it has been demonstrated that a simple modification as the protonation of the imino nitrogen atoms of porphyrin ring brings about changes in properties such as hydrophobicity, membrane binding, passive diffusion through the plasmatic membrane, photodynamic effect, and photophysical properties [19–22]. Additionally, it has also been demonstrated that the protonation of porphyrins has a potential application in optical limiting due to the enhancement of the reverse saturable absorption process [23,24].

In general, the protonation of the imino nitrogen atoms of porphyrin rings occurs in acidic environment and can influence the way the sensitizers are internalized and retained in a cell [25]. In live systems, it is known that pH can vary in different compartments of the cell and tissues to maintain the normal functionality of the organisms [26]. Usually, diseased cells and tissues, for instance cancer cells, present an acidic microenvironment due to glycolytic cancer metabolism, hypoxia, and deficient blood perfusion [27,28]. In turn, this difference in proton concentration could certainly influence the effectiveness of sensitizers administered as a medicine in photodynamic therapy (PDT), or other applications. Consequently, to rationalize the use of porphyrin molecules in PDT applications, it is imperative to investigate the changes of their photophysical properties in acidic media.

The aim of our study was to evaluate the effect of pH on the electronic properties of sulfonated halogenated porphyrins. To achieve such task, we analyzed the effects of pH and protonation, using absorption, fluorescence emission and NMR spectroscopies, and quantum mechanical calculations based on Density Functional Theory. The present study is an essential contribution in order to understand the properties of these molecules and probe their potential for applications such as photodynamic therapy and photonics.

Materials and methods

The sulfonated halogenated porphyrins, 5,10,15,20-tetrakis (2-chloro-5-sulfophenyl)porphyrin (TC), 5,10,15,20-tetrakis (2-fluoro-5-sulfophenyl)porphyrin (TF), 5,10,15,20-tetrakis(2,6-dichloro-3-sulfophenyl)porphyrin (TDC) and 5,10,15,20-tetrakis(2,6-difluoro-3-sulfophenyl)porphyrin (TDF) were synthesized by previously reported methods [16,17]. Their structures are presented in Fig. 1. All porphyrins employed in this work were dissolved in Milli-Q quality water without further purification, and pH values were adjusted by addition of appropriate amounts of HCl or NaOH stock solutions.

The UV–Vis absorbance spectra were obtained with a Beckman DU 940 spectrometer, and the fluorescence spectra were obtained with Fluorolog-3 spectrofluorometer – Horiba/Jobin–Yvon Inc. The concentrations were monitored spectrophotometrically and all photophysical measurements were performed in aqueous solution, at room temperature. The fluorescence quantum yield (φ_f) was obtained by comparison between the integrated emission spectra of the investigated and reference sample, which was aqueous solution of *meso*-tetrakis(4-N-methyl-pyridiniumyl) porphyrin (TMPyP) in its free base form at pH 6.8 [29].

Fluorescence decay profiles were obtained using an apparatus based on the time correlated single photon counting method. The excitation source was a titanium–sapphire laser (Tsunami 3950–Spectra Physics), pumped by the second harmonic of a diode-pumped Nd:YVO_4 laser (Millenia–Spectra Physics) and the frequency doubled to 465 nm in a LBO crystal (GWN-23PL–Spectra Physics). Pulse repetition rate was set to 8.0 MHz and excitation pulses were directed to a L-format Edinburgh FL900 spectrometer. Single photons were detected by a cooled Hamamatsu R3809U microchannel-plate photomultiplier yielding an instrument response function of ~100 ps.

^{1}H NMR spectra were recorded with a Bruker Avance III 11.75 Tesla spectrometer at 298 K using a 5 mm inverse probe head with a z-gradient. The spectra were obtained at 500.13 MHz for ^{1}H, using 10 μs as the 90° pulse width. Solutions of halogenated sulfonated porphyrins were prepared, with pH 0.5 and 7.0, using D_2O as solvent, for protonated and nonprotonated solutions, respectively.

Concerning quantum chemical calculations, the isolated and nonprotonated porphyrin had its ground state structure optimized and vibration frequencies calculated using the CAM-B3LYP hybrid functional [30]. Subsequently, the outlying groups as well as extra protons were inserted, the singlet ground state structure of the compounds under study was optimized and vibration frequencies calculated, also using the CAM-B3LYP hybrid functional in a SCRF approach based on the application of the IEFPCM model [31,32], used to build a continuum of uniform dielectric constant corresponding to water. All calculations used the 6-31G(d,p) basis set and were made without any symmetry constraints; geometries were confirmed as stationary structures by the presence of only real vibration frequencies.

The singlet–singlet transitions and the corresponding oscillator strengths were calculated from the optimized structures using the same hybrid functional in a TD-DFT approach, using the 6-311G(d,p) basis set and the IEFPCM model. All calculations were made using Gaussian 09 [33], and Gauss View 5.0.8 [34] was employed in the analysis of the results.

Results and discussion

Absorption spectra

Typically, the UV–Vis absorption spectra of porphyrins present two main bands in UV–Vis region [35]. The B-band (also called Soret band) generally located between 380 and 450 nm, and the Q bands between 500 and 650 nm [44]. Usually, the four lowest absorption bands of nonprotonated porphyrins are referred to, in the order of rising transition frequency, as $Q_x(0,0)$, $Q_x(1,0)$, $Q_y(0,0)$, and $Q_y(1,0)$.

Fig. 2(a) and (b) presents the changes in the absorption spectra of TF and TDF porphyrins with different pH values, from 12.0 to 0.4, at room temperature. Fig. 2(c) and (d) show the variation of magnitude for the higher intensity of Q bands of nonprotonated samples ($Q_y(1,0)$), with different pH values. The spectral changes observed have been assigned to the protonation of porphyrin molecules in solution [36–38]. The p*K* values can be estimated through a sigmoidal fitting of Fig. 2(c) and (d). The results show that porphyrins bearing one halogen atom at the phenyl ring (TC and TF), present similar p*K* values, ~3.5, while for porphyrins with two halogen atoms (TDC and TDF) at phenyl moieties the p*K* values were reduced to 2.1 (TDC) and 2.3 (TDF). Aiming at analyzing the spectral changes caused by protonation, we chose, for TC and TF, to work with pH values equal to 1.0 and 7.0, while for TDC and TDF we used pH values equal to 0.5 and 7.0.

For the analysis of protonation effects on the characteristics of halogenated sulfonated porphyrins, we used the free base 5,10,15,20-tetrakis(4-sulfophenyl) porphyrin (TPPS) as a reference compound, whose photophysical properties and pH dependence are very well characterized [24,38]. It is known that TPPS, with no halogen atoms in its structure, presents a p*K* value close to 4.5, showing that the presence of halogen atoms decreases the