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Protonation/deprotonation process of Emodin in aqueous solution and pK_a determination: UV/Visible spectrophotometric titration and quantum/molecular mechanics calculations

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ABSTRACT

We combined theoretical and experimental studies to elucidate the important deprotonation process of Emodin in water. We used the UV/Visible spectrophotometric titration curves to obtain its pK_a values, $pK_{a1} = 8.0 \pm 0.1$ and $pK_{a2} = 10.9 \pm 0.2$. Additionally, we obtained the pK_a values of Emodin in the water–methanol mixture (1:3v/v). We give a new interpretation of the experimental data, obtaining apparent $pK_{a1} = 6.2 \pm 0.1$, $pK_{a2} = 8.3 \pm 0.1$ and $pK_{a3} > 12.7$.

Performing quantum mechanics calculations for all possible deprotonation sites and tautomeric isomers of Emodin in vacuum and in water, we identified the sites of the first and second deprotonation. We calculated the standard deprotonation free energy of Emodin in water and the pK_{a1}, using an explicit model of the solvent, with Free Energy Perturbation theory in Monte Carlo simulations obtaining, $\Delta G_{aq} = 12.1 \pm 1.4$ kcal/mol and $pK_{a1} = 8.7 \pm 0.9$. With the polarizable continuum model for the solvent, we obtained $\Delta G_{aq} = 11.6 \pm 1.0$ kcal/mol and $pK_{a1} = 8.3 \pm 0.7$. Both solvent models gave theoretical results in very good agreement with the experimental values.

1. Introduction

Emodin (1,3,8-trihydroxy-6-methyl-9,10-anthraquinone, Fig. 1) is one of the most abundant anthraquinone derivatives found in nature [1]. It is the active principle of herbal medicines deriving from Polygonaceae, Rhamnaceae and Cassieae [2]. This anthraquinone is known to have biological activity, such as anti-bacterial [3–5], antiviral [6,7], anti-inflammatory [8,9], anti-cancer activities [10–12] and virucidal agent [2].

The UV/Visible spectrum of the Emodin has been used to study its interaction with the biological environment, like DNA [13,14] and human serum albumin (HSA) [15], and also as a spectrophotometric reagent for detection of various metal ions [16]. Emodin is a yellow amorphous solid, insoluble in water at acidic *pH*, but red and soluble in water at alkaline *pH*. In acidic aqueous solution and in common organic solvents, there is a broad UV/Visible absorption band between 350 and 500 nm in the Emodin spectrum with a λ_{max} around 440 nm that is responsible for the yellow color of these solutions. In alkaline solutions, this broad band is red shifted to 450–600 nm with a λ_{max} varying between 520 and 555 nm depending on solvent and yielding a red color to these solutions. The UV/Visible absorption spectra and the first band λ_{max} of Emodin in several solvents in acidic and alkaline *pH* are presented in the Supplementary Material. It is known that this noticeable change in color of Emodin in solution with different *pH* is due to a deprotonation process [16]. The neutral form of the Emodin (EMH) is yellow and the anionic/deprotonated form (EM^-) is red. Based on the potentiometric titration of the Emodin in a methanol–water mixture, Pal and Jana [16] established the one-proton dissociation equilibrium in the range *pH* 2–10 and determined an apparent pK_{a1} of 7.2 in this mixture. They also suggested a stepwise dissociation of three protons: first in position 3 (3-oxido-6-methyl-1,8-dihydroxy-9,10-anthraquinone), second in position 8 (3,8-oxido-6-methyl-1-hydroxy-9,10-anthraquinone) and third in position 1 (1,3,8-oxido-6-methyl-9,10-anthraquinone). An experimental and theoretical investigation of the electronic transitions of Emodin and its conjugated base with deprotonation at position 3 in ethanol has been published [17]. They used synchrotron linear dichroism spectroscopy and quantum mechanics calculations with Density Functional Theory (TD-B3LYP/6-31+G(d,p)) to characterize the absorption spectrum of Emodin. In their calculation, the solvent was included using the polarizable continuum model (PCM) and one additional explicit solvent molecule hydrogen bonded to the deprotonated oxygen of Emodin.

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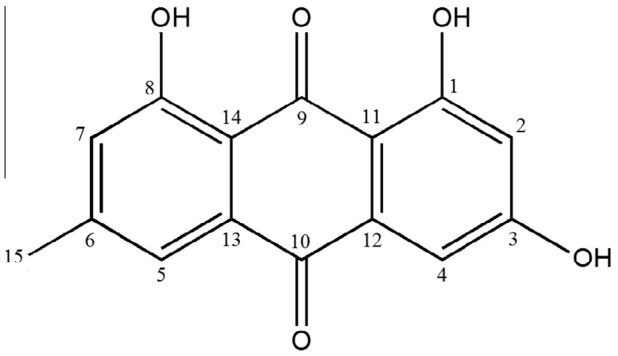

Fig. 1. Chemical structure and atomic numbering of Emodin in its neutral form (EMH).

The protonation/deprotonation process of a pharmacophore is a topic of large interest in physics, chemistry and pharmaceutical industry, because it can change the pharmacological activity of a compound [18,19]. Frequently, a well-defined acidity constant (pK_a) describes the chemical reactivity of molecules [20]. Hence, the determination of the value of the pK_a has been the object of many experimental and theoretical studies. The experimental methods commonly used for the determination of the pK_a are: dissolution rate method [21], ionophoretic techniques [22], isotachophoresis [23], conductivity [24], potentiometric titration [25], nuclear magnetic resonance spectroscopy [26], UV/Visible spectrophotometric titration [27], thermodynamics [28], Z-scan technique [29] and capillary electrophoresis [30]. On the other hand, the theoretical works have utilized computer simulations with explicit solvent models and Free Energy Perturbation [31–34], Poisson–Boltzmann equation with continuum solvent models [35,36], quantum mechanics calculation associated with continuum solvent models [37–42] and cluster based quantum mechanics calculation [43].

The protonation/deprotonation processes of Emodin in organic solutions were experimentally analyzed, and acidity constants of Emodin were determined in water–methanol [16], water–ethanol [17], water–acetonitrile and acetonitrile [30]. However, to our knowledge, experimental and theoretical studies of the Emodin in aqueous solution have never been reported. This is a non-trivial task, due to the low solubility of the Emodin in acidic aqueous solution [16].

In the present work, we study the protonation/deprotonation process of Emodin in aqueous solution, using experimental and theoretical techniques. With the UV/Visible spectrophotometric titration technique, we determined the pK_a of Emodin in water. The solubility difficulty was minimized by titrating the Emodin in aqueous solution from alkaline to acidic pH (from 13.8 to 2.0), and measuring the UV/Visible absorption spectrum immediately after strongly vortexing the sample.

Additionally to the experimental assay, we performed a theoretical study to identify the position of the first deprotonation of Emodin in aqueous solution. The theoretical approach was based in thermodynamic cycles obtained from two different equilibrium reactions, $XH \Longleftrightarrow X^- + H^+$ and $XH + H_2O \Longleftrightarrow X^- + H_3O^+$, where the XH is the molecule of interest and the reactions were investigated in gas phase and in aqueous solution. These thermodynamic cycles were previously used [39,40,44,45] to calculate the deprotonation free energy of the XH in aqueous solution (ΔG_{aq}), and additionally the pK_a. The values of the ΔG_{aq} were calculated using an expression, obtained from the thermodynamic cycle, that relates ΔG_{aq} with the free energy of deprotonation in gas phase (ΔG_g) and the relative free energies of solvation ($\Delta\Delta G_{solv}$) of the neutral and ionic species involved in the reactions ($\Delta G_{aq} = \Delta G_g + \Delta\Delta G_{solv}$).

The ΔG_g was computed by quantum mechanical calculations, using Density Functional Theory (DFT) and Møller–Plesset second order perturbation theory (MP2). All the possibilities of the first deprotonation form and the tautomeric isomers of the Emodin in gas phase and in aqueous solution were analyzed. We identified that the first deprotonation at position 3 and the second deprotonation at position 8 are the most favored situation. This result is in agreement with the Pal and Jana suggestion [16]. The solvation free energies of each Emodin species, ΔG_{solv}(EMH) and ΔG_{solv}(EM^-), were calculated using Monte Carlo (MC) simulations combined with Free Energy Perturbation theory (FEP) and the ΔG_{aq} values were obtained using the two thermodynamic cycles. Finally, the theoretical value for the pK_{a1} was obtained and it presents a very good agreement with the experimental data.

2. Experimental

2.1. Materials

Emodin ($C_{15}H_{10}O_5$, Fig. 1), Hydrochloric acid (HCl), Sodium hydroxide (NaOH) and Methanol (CH_3OH) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA) and used without further purification. Milli-Q water was used throughout.

2.2. Sample preparation

A 10 mM Emodin stock solution was prepared in ethanol-methanol mixture at 4:1 v/v. Aliquots of this stock solution were separated in glass vials, dried under a stream of N_2, and left under reduced pressure for a minimum of two hours, to remove traces of organic solvents. The Emodin film so formed was dissolved in water at two different concentrations, 0.1 and 0.025 mM, and in water–methanol mixture (1:3 v/v) at concentration of 0.025 mM. For each concentration, two different pH samples were prepared at room temperature: an alkaline at $pH \sim 13$, and an acidic at $pH \sim 2$, by the addition of NaOH and HCl, respectively. In water, at $pH \sim 2$ the Emodin aggregates, and precipitates after a minute, hence the sample had to be strongly vortexed before used.

2.3. UV/Visible spectrophotometric titration

Absorbance measurements were performed with a Varian Cary 50 UV/Visible Spectrophotometer, at room temperature. Samples were placed in quartz cuvettes with 10 mm optical pathway. Absorbance measurements of the Emodin solution samples were performed from alkaline to acidic pH values, by successive addition of small aliquots (around 5 μL) of the Emodin acidic solution (1:500 v/v of HCl). For the two studied concentrations, two independent samples were prepared for each pH and around 70 UV/Visible spectra were measured in the pH interval, from ~13 to 2. The samples were homogenized by strongly vortexing immediately before each measurement and its pH was measured with a Mettler Toledo pH-meter. Therefore, in the case of the water–methanol mixture the pH presented are the apparent pH values.

2.4. Determination of acidity constant

The deprotonation probability of a single site in a solute molecule is given by Eq. (1), algebraically equivalent to the Henderson–Hasselbalch (HH) equation, describing an increasing sigmoidal standard titration curves:

$$\xi = \frac{10^{(pH-pK_a)}}{(1+10^{(pH-pK_a)})} \quad (1)$$