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Photophysical aspects of biological photosensitizer Kynurenic acid from the perspective of experimental and quantum chemical study

Anuva Samanta [a], Nikhil Guchhait [b,*], Subhash Chandra Bhattacharya [a,*]

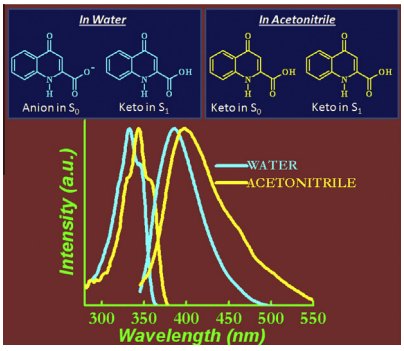
[a] Department of Chemistry, Jadavpur University Raja S.C. Mallick Road, Kolkata 700 032, India
[b] Department of Chemistry, University of Calcutta 92, A.P.C. Road, Kolkata 700 009, India

HIGHLIGHTS

- Kynurenic acid (KA), an endogeneous and neuroactive NMDA receptor antagonist.
- KA is an end product of "*kynurenine pathway*".
- Spectral ad theoretical studies of KA.
- In S0 state, KA exists mainly in anionic form in water.
- The presence of keto tautomer in S1 state.

GRAPHICAL ABSTRACT

Existence of various species such as keto, enol, anion of Kynurenic acid, a well-recognized antiexcitotoxic and anticonvulsant drug, and the byproduct of tryptophan metabolism has been established based on the steady state and time resolved absorption and fluorescence spectroscopy. Quantum chemical calculations support the experimental findings.



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ABSTRACT

In the present contribution, we have explored ground and excited state spectroscopic properties of an antiexcitotoxic and anticonvulsant drug, Kynurenic acid (KA), through steady-state absorption, emission and time-resolved emission spectroscopy and quantum chemical calculations. The main focus of this article is to illustrate the effects of various parameters such as the nature of the solvents and pH of the medium on the spectral properties of KA which confirms the presence of different neutral and ionic species in the ground and excited states. The molecule KA exists mainly as keto- or anionic form in the ground state, whereas the main contribution of its emission is arising from the keto tautomer in the excited state. Quantum chemical calculations by Density Functional Theory (DFT) method has been effectively employed to correlate the experimental findings. The ground and excited state properties of KA ascertained by means of experimental and theoretical method reveal that it resembles well with other two compounds, 4-hydroxyquinoline and xanthurenic acid formed by the decomposition of UV filters.

* Corresponding authors. Tel.: +91 33 2350 8386; fax: 91 033 2351 9755 (N. Guchhait). Tel.: +91 033 2414 6223, fax: +91 033 24146584 (S.C. Bhattacharya).
E-mail addresses: nguchhait@yahoo.com (N. Guchhait), sbjuchem@yahoo.com (S.C. Bhattacharya).

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Introduction

Human lens and retina are protected from photodamage [1,2] by molecular UV filters consisting of low molecular weight compounds present in the lens. In fact, the UV radiation from solar light that finally reaches our eyes after transmission through the Earth's sunscreen, i.e., the ozone layer, still contains ultraviolet components in the wavelength 300–400 nm, which may be detrimental to our eyes. But UV filters, present in the human lens, absorb UV light from 300 to 400 nm wavelength region, thereby protecting our eyes from UV-induced damage [1,3,4]. The well-known UV filters, namely kynurenine, 3-hydroxykynurenine and 3-hydroxykynurenine O-β-D-glucoside are originated as the product of enzymatic metabolism of amino acid, L-tryptophan [1,5–7]. These compounds can undergo spontaneous deamination [8] during photochemical, thermal and enzymatic reaction yielding highly reactive species which are capable to make important modification of lens protein, like nuclear cataract development [9–11]. The decomposition of UV filters under physiological conditions gives rise to the formation of different compounds. For example, thermal, photochemical or enzymatic reactions of kynurenine can produce 4-hydroxyquinoline (4HQN), 4,8-dihydroxyquinoline-2-carboxylic acid (xanthurenic acid, XA) and 4-hydroxyquinoline-2-carboxylic acid, commonly known as Kynurenic acid (KA). In 1853, KA was first identified when it was spotted in canine urine [12]. Half a century later, in 1904, the compound was recognized as a byproduct of tryptophan degradation [13]. KA is the end product in one of the branches of the "*kynurenine pathway*". KA can be formed from kynurenine either through spontaneous deamination forming 4-(2-aminophenyl)-2,4-dioxobutanoic acid followed by cyclization, or under the action of an enzyme kynurenine aminotransferase [14,15]. KA is a renowned endogenous antagonist of the glutamate ionotropic excitatory amino acid receptors N-methyl-D-aspartate (NMDA) [16,17]. Till date, among different endogenous NMDA receptor antagonist, only KA can mediate glutamatergic hypofunction. Despite the NMDA receptor antagonism, KA can act as a nicotinic receptor antagonist [18]. The involvement of KA in the pathophysiology of psychiatric disorders, including schizophrenia, has been widely reported [19–21]. KA has not been found in normal lenses or its concentration is too below the detectable level. Actually, KA is chemically or photochemically much more reactive than that of other UV filters, so after formation, it channelizes itself to some other reaction thereby lowering its abundance in lens.

The present work is devoted to the exploration of the photophysical properties of KA in different solvents, pH etc. The important characteristics of various hydroquinolines, like 4HQN, XA, are that depending on the nature of the solvents, they can exist in the enol and keto tautomeric forms (E and K), and also undergo protonation and deprotonation reactions with the variation of the pH of the medium [22,23]. Similar to 4HQN and XA, KA can also exist as four different species, K, E, cation and anion, depending upon the acidity of the aqueous solution as reported by Pileni et al. [24,25] and Zelentsova et al. [26]. Besides aqueous environment, up to now, the effect of different solvent-assisted tautomerization has not been taken under consideration. Therefore, for a better understanding of the ground and excited state properties of KA, we have employed electronic absorption and fluorescence spectroscopy to determine the energetically favorable forms of the target molecule in different solvents and with variation of pH of the medium. Effect of different surfactants on this molecule may also ascertain the most probable forms of KA in aqueous solution. So far our knowledge goes to, there are no detailed theoretical study on this molecule as was done for 4HQN and XA [22,23]. Quantum chemical calculations have been performed on the structural aspect of the molecule to correlate with experimental findings.

Experimental section

Materials

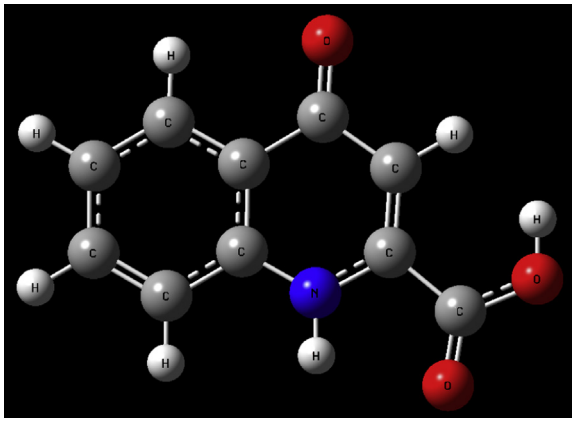
Kynurenic acid (Scheme 1) was used as received from Sigma–Aldrich without further purification. Water was triply distilled for the preparation of aqueous solutions. Spectroscopic grade organic solvents methylcyclohexane (MCH), tetrahydrofuran (THF), 1,4-dioxane (DOX), chloroform ($CHCl_3$), acetonitrile (ACN), dichloromethane (DCM), dimethylsulfoxide (DMSO), isopropanol (iPrOH), 1-butanol (BuOH), ethanol (EtOH) and methanol (MeOH) were purchased from Spectrochem (India) and the purity of solvents have been verified by measuring their spectra in the wavelength range used. Sodium hydroxide (NaOH) and sulfuric acid (H_2SO_4) were obtained from Merck and triethylamine (TEA) from Spectrochem (India) was used as received. The surfactants sodium dodecylsulphate (SDS), cetyltrimethylammoniumbromide (CTAB) and p-tert-octylphenoxy polyoxyethanol (Triton X-100, TX-100) were purchased from SRL, Spectrochem and Merck, respectively and used as received.

Instrumentations and methods

The steady state absorption and emission spectra of KA in different medium were acquired on Hitachi UV–Vis U-3501 spectrophotometer and PerkinElmer LS-55 fluorimeter, respectively, with quartz cuvettes of 1 cm path length. In all measurements, the sample concentration has been maintained within the range of 10^{-5}–10^{-6} mol/dm^3 in order to avoid aggregation and self quenching phenomena. All experiments were carried out at room temperature (298 K). ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were taken in d_6-DMSO using tetramethylsilane as an internal standard on a Bruker AV 3000 supercon spectrometer (300 MHz); chemical shift are in δ units (ppm). FT-IR measurements were carried out at room temperature using Perkin–Elmer Spectrum-100 spectrophotometer using the KBr wafer technique. The pH of different solutions was measured using μ-pH system 361 pH meter from Systronics, India.

Fluorescence quantum yield (Φ_F) of KA in different solvents has been achieved based on the secondary standard method using β-napthol (Φ_F = 0.23 in MCH) as the secondary standard and the following equation [27–29] has been used for this estimation:

$$\Phi_F = \Phi_F^0 \cdot \frac{n^2}{n_0^2} \cdot \frac{OD^0}{OD} \cdot \frac{\int I_f(\lambda_f) d\lambda_f}{\int I_f^0(\lambda_f) d\lambda_f}. \quad (1)$$



Scheme 1. Optimized structure of Kynurenic acid (KA) at DFT/B3LYP/6-31++G** level.