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Acid-alkaline properties of triplet state and radical of kynurenic acid

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ABSTRACT

Kynurenic acid (KNA), a degradation product of endogenous UV filter of the human lens kynurenine, is a convenient model compound for studying reactions of photoexcited chromophores with amino acids, peptides and proteins. In this work, transient absorption spectra of triplet KNA (^{T}KNA) and KNA radical (KNA$\cdot^{-}$) were obtained for aqueous solutions with different pH's, and the dissociation constants for ^{T}KNA (pKa = 3.7) and KNA$\cdot^{-}$ (pKa = 5.5) have been determined. The quenching of ^{T}KNA by oxygen, tryptophan and ascorbate proceeds with nearly diffusion rate constants, weakly dependent on the charge on ^{T}KNA and on quencher. The quenching by tyrosine and histidine are much slower, and the quenching rate constants depend on solution pH's. The most pronounced pH-dependence was found for ^{T}KNA quenching by thiols cysteine and glutathione, which can be attributed to the switch of the quenching mechanism from the electron transfer in alkaline solutions to the hydrogen atom transfer under neutral and acidic conditions.

1. Introduction

Solar radiation is the major source of biological energy on the Earth; at the same time, excessive solar irradiation (especially UV light) may disrupt many metabolic processes and inflict photochemical damages in biological tissues. In particular, UV radiation may cause protein photooxidation, coloration and aggregation [1–4], leading to the development of human diseases, such as cataracts [5,6] and skin cancer [7–9]. The skin and eye protection against harmful UV radiation is provided by natural UV filters (melanins in the skin [10] and kynurenines (KNs) in the lens [11–14]), which absorb UV light and direct the absorbed energy into benign channels. However, the natural UV filters under UV radiation can also generate reactive species and cause phototoxic, degenerative, and cancerogenic effects [15–18]. Therefore, the study of reactions between photoexcited chromophores and biological substances is important for understanding the mechanisms of UV light-induced human diseases.

Unfortunately, the reactions with participation of molecular UV filters are difficult to study: the quantum yield of reactive species (triplet states and radicals) is very low, and the products of UV filter photodecomposition are photochemically much more active than the initial compounds [19–21]. Thus, the secondary photochemistry starts to play an important role already at the early stages of photolysis. For that reason, the use of model compounds is preferable. An optimal model compound should have the chemical structure similar to that of natural UV filters, absorb in UV-A region (315–400 nm), and demonstrate high triplet yield. Recently, the use of kynurenic acid (KNA) for studying biologically-related photochemical reactions [4,22] has been proposed. KNA is the product of decomposition of molecular UV filter KN, present in the human lens. In neutral aqueous solution it has an absorption maximum at 332 nm, the triplet quantum yield is approximately 80%, and the reactivity of the KNA triplet state toward aromatic amino acids and other compounds is similar to that of KN triplet state [4,21].

It is important to notice that although the concentration of KNA in the human lens is lower than that of KN by 2–3 orders of magnitude [14,23–25], the KNA triplet yield is approximately 100 times higher than that of KN [18,21,26]. In cataractous lenses, the level of KNA significantly increases [25], and the impact of photochemical reactions of KNA on the lens proteins becomes comparable with that of abundant but photochemically inert KN and other UV filters. Thus, KNA is not only a convenient model compound, but its photochemical reactions are biologically important.

The present work is aimed at the study of pH dependence of spectral and chemical properties of short-lived intermediates formed under KNA irradiation by UV-A light. The major goal of the study are to obtain the electronic spectra of triplet ^{T}KNA and radical KNA$\cdot^{-}$ at different pH values, to determine the pKa values of the triplet state and radical, and to measure the rate constants of ^{T}KNA reaction with biologically-related compounds (amino acids, antioxidants, oxygen) at different pH's.

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2. Experimental

All chemicals used in this work were of highest purity from either Amersham Biosciences (Amersham, UK) or Sigma (St. Louis, MO). Phosphate salts (AppliChem, Germany) and deionized water (18.2 MOhm) were used for 150 mM phosphate buffered saline (PBS) preparation.

Transient absorption spectra and kinetics were measured with the use of a nanosecond laser flash photolysis (LFP) setup described earlier [18]. Briefly, samples in a 10 × 8 mm^2 quartz cell were irradiated with a Quanta-Ray LAB-130-10 Nd:YAG laser from SpectraPhysics (Mountain View, CA, USA): 355 nm, pulse duration 8 ns, pulse energy up to 135 mJ. A fraction of the laser beam was split by a quartz plate and directed to a photodiode for triggering an oscilloscope and to a Newport 1918-C power meter (Franklin, MA, USA) for the permanent monitoring of the laser energy. The dimensions of the laser beam at the front of the cell were reduced by a diaphragm to 2.5 mm × 8 mm. The monitoring system includes a DKSh-150 xenon short-arc lamp from Stella Ltd (Moscow, Russia) connected to a high current pulser, a Newport 78025 monochromator (Stratford, CT, USA), a 9794B photomultiplier from ET Enterprises Ltd (Uxbridge, UK), and a WaveRunner 104MXi digital oscilloscope from LeCroy (Chestnut Ridge, NY, USA). The probe light, concentrated in a rectangle of 2.5 mm height and 1 mm width, passed through the cell parallel to the front (laser irradiated) window. Thus, in all experiments the excitation optical length was 1 mm, and the monitoring optical length was 8 mm. All solutions were bubbled with argon for 15 min prior to and during irradiation, unless stated otherwise.

3. Results

3.1. Spectra of the KNA triplet state

Earlier, it has been shown that in aqueous solution KNA is present in the anionic form in the pH range from 2.5 to 11.6 [21] (Scheme 1). It has an absorption maximum at 332 nm ($\varepsilon_{332} = 1.0 \times 10^4\ M^{-1}\,cm^{-1}$), and the KNA absorption coefficient at the excitation wavelength is $\varepsilon_{355} = 3.0 \times 10^3\ M^{-1}\,cm^{-1}$. The irradiation of KNA in neutral aqueous solution results in the formation of KNA triplet state with the quantum yield of approximately 80% [21]. The triplet state has absorption maxima at 270 nm and 600 nm, and in the absence of triplet quenchers, it decays mainly via the second-order reaction of triplet-triplet annihilation with $k_2/\varepsilon = 4.6 \times 10^5$ cm s^{-1} for 600 nm and $k_2/\varepsilon = 2.7 \times 10^5$ cm s^{-1} for 290 nm [21]. In the present work, the triplet spectra were measured for 0.3 mM KNA solutions in PBS for three pH values – pH 2.8, pH 7.3, and pH 10.2, the results are presented in Fig. 1. The spectra obtained for neutral and basic solutions practically coincide and are in a good agreement with the previously published spectrum [21], while under acidic conditions the maximum undergoes blue shift to 540 nm, and its intensity significantly decreases. This observation indicates that the pKa value of ^{T}KNA lies between 2.8 and 7.3. The titration curve (Fig. 2) was obtained by measuring the triplet absorption at 600 nm observed immediately after the 355 nm laser pulse at different pH values. It was presumed that no other species but KNA triplet state contribute into the transient absorption at 600 nm, and the equilibrium between the protonated T_p and deprotonated T_d forms of the triplet state during the registration is already established:

$$Tp = T0 \times \frac{[H^+]}{[H^+] + K_a}$$

$$Td = T0 \times \frac{K_a}{[H^+] + K_a}$$

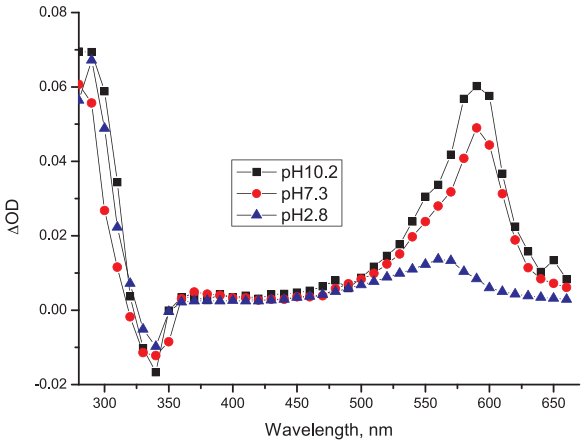
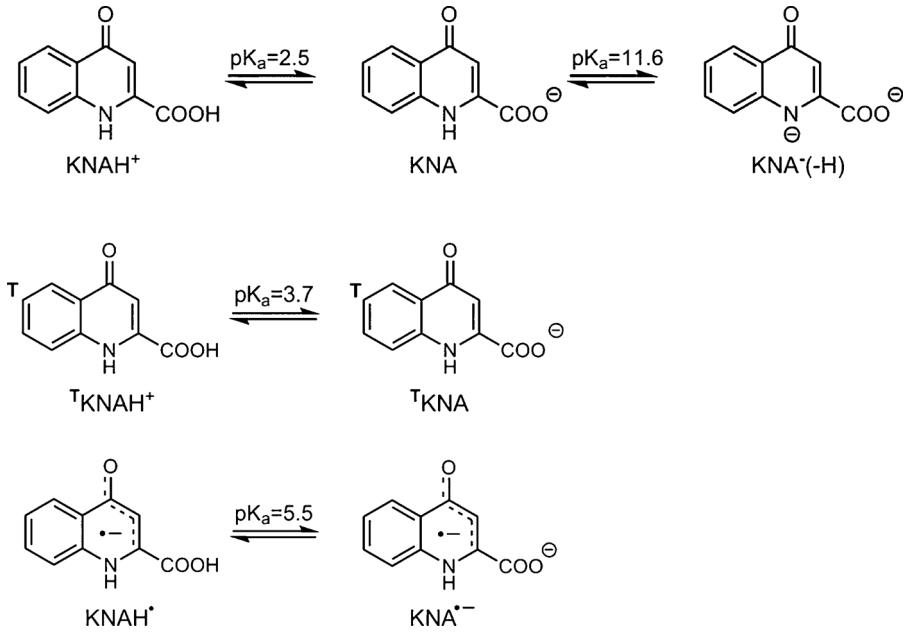


Fig. 1. Transient absorption spectra observed 250 ns after the laser pulse irradiation of 0.3 mM aqueous KNA solutions under argon at pH 10.2 (squares), 7.3 (circles), and 2.8 (triangles).



Scheme 1. Dissosiation of ground-state KNA, triplet ^{T}KNA, and radical KNA$^{\bullet -}$.