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Biphotonic ionization of kynurenine and 3-hydroxykynurenine

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Abstract

The photoionization of kynurenine (KN) and 3-hydroxykynurenine (3OHKN) in aqueous solutions proceeds via a biphotonic mechanism. The precursors for ionization are the triplet states ^{T}KN and ^{T}3OHKN, absorbing the second light quantum. The addition of solvated electrons to the parent molecules with the rate constant 2.0×10^{10} M^{-1} s^{-1} results in the formation of anion radicals, the latter in neutral solutions undergoes fast protonation. The individual spectra of all intermediates – triplet states, cation radicals, electron adducts – formed under UV irradiation of KN and 3OHKN are obtained.

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1. Introduction

A group of tryptophan-derived compounds: kynurenine (KN), 3-hydroxykynurenine (3OHKN), 3-hydroxykynurenine glucoside (3OHKG), and 4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid glucoside (AHBG), are found in the human lens, where they absorb UV light in the 300–400 nm region protecting the lens and retina from light-induced tissue damage [1–5]. Three of these compounds, KN, 3OHKN and 3OHKG, have been shown to be chemically unstable: at physiological pH and temperature they can undergo deamination or decarboxylation [6,7]. The products so formed, carboxyketoalkenes and aminoketoalkenes, are highly reactive species which can bind to lens proteins [6,8–12]. These reactions can result in the eventual accumulation of the modified proteins in the lens and to the development of a cataract [13,14].

An alternative channel of lens protein modification is the photochemical reactions of the UV filter compounds [15–19]. Kynurenine and most of its derivatives are weak photosensitizers. It has been reported that KN and 3OHKN do not photosensitize the formation of singlet oxygen and superoxide [20], and that the first singlet exited states of these compounds decay to the ground state in picosecond time scale [21], directing light energy into benign channels. Nevertheless, in model experiments the reactions of photoexcited KN with some biological compounds have been observed [22]. In our recent publication [23] we revealed the formation of the KN triplet state under UV irradiation. At physiological pH the quantum yield of the triplet state formation is relatively low, about 2%, and yet this route may be the primary step for the subsequent reactions, resulting in the irreversible chemical modification of the lens proteins.

The present work is aimed at the study of one more potentially harmful photochemical reaction of KN and 3OHKN, photoionization. This reaction is known for aromatic amino acids in aqueous solutions: tryptophan, tyrosine, phenylalanine [24–26]. The intermediates formed – solvated electron and cation radical – can react with other molecules. In particular, the photoionization of tryptophan is one of the major pathways of photo-oxidation of many proteins [27]. The main goals of this work are to demonstrate that photoionization takes place in the photolysis of KN and 3OHKN, to determine the precursor for ionization, and to characterize the short lived intermediates formed under photolysis of these compounds.

2. Experimental details

A detailed description of the LFP equipment has been published earlier [28,29]. Solutions, running through a rectangular cell (inner dimensions 3 mm × 10 mm), were irradiated with a

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Quanta-Ray LAB-130-10 Nd:YAG laser (pulse duration 8 ns; 355 nm, pulse energy up to 150 mJ; 266 nm, pulse energy up to 70 mJ). The dimensions of the laser beam at the front of the cell were 2.5 mm × 8 mm. The monitoring system includes a DKSh-150 xenon short-arc lamp connected to a high current pulser, a home-made monochromator, a 9794B photomultiplier (Electron Tubes Ltd.), and a LeCroy 9310A digitizer. The monitoring light, concentrated in a rectangle of 2.5 mm height and 1 mm width, passed through the cell along 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.

Actinometry was performed using naphthalene in cyclohexane. The incident laser energy was determined by triplet naphthalene absorption at 414 nm (absorption coefficient $2.45 \times 10^4\ M^{-1}\ cm^{-1}$, triplet quantum yield 0.75 [30,31]).

D,L-Kynurenine and 3-hydroxy-D,L-kynurenine (Sigma/Aldrich) were used as received. Solutions were prepared with the use of phosphate buffers.

3. Results and discussion

Transient absorption spectra, obtained immediately after the 355 nm laser irradiation in the photolysis of Ar-saturated aqueous solutions of KN (2×10^{-4} M) and 3OHKN (2.6×10^{-4} M), are shown in Figs. 1 and 2 by open triangles. A promptly decaying signal on the right-hand side of the spectra was attributed to the solvated electron. Firstly, the spectrum of this intermediate is similar to that of solvated electron; secondly, this signal is readily quenched by well-known electron scavengers, N_2O and acetone. The decay of the signal is monoexponential, the observed decay rate constants k_{obs1} (KN photolysis) and k_{obs2} (3OHKN photolysis) are proportional to the concentration of the initial compounds:

$$k_{obs1} = k_{e1} \times [KN], \quad k_{obs2} = k_{e2} \times [3OHKN]$$

Thus, in our experimental conditions the main channel of solvated electron decay is the attachment to the initial compound. The observed rate constants k_{obs1} and k_{obs2} were measured for the different concentrations of the initial compounds, the calculated rate constants for the electron addition to KN and 3OHKN are $k_{e1} = (2.0 \pm 0.2) \times 10^{10}\ M^{-1}\ s^{-1}$ and $k_{e2} = (1.9 \pm 0.2) \times 10^{10}\ M^{-1}\ s^{-1}$, respectively, which is in a good agreement with the values obtained by pulse radiolysis [32].

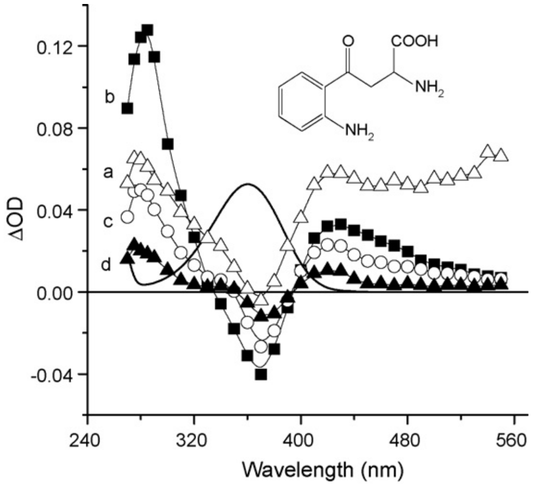

Fig. 1. Transient absorption spectra, obtained in 355 nm photolysis of 2×10^{-4} M KN–(a) open triangles: under Ar, 50 ns after the laser pulse; (b) solid squares: under Ar, 1 μs after the laser pulse; (c) open circles: under Ar in the presence of 5×10^{-3} M acetone, 1 μs after the laser pulse; (d) solid triangles: under O_2 in the presence of 5×10^{-3} M acetone, 1 μs after the laser pulse. Solid line shows the absorption spectrum of KN.

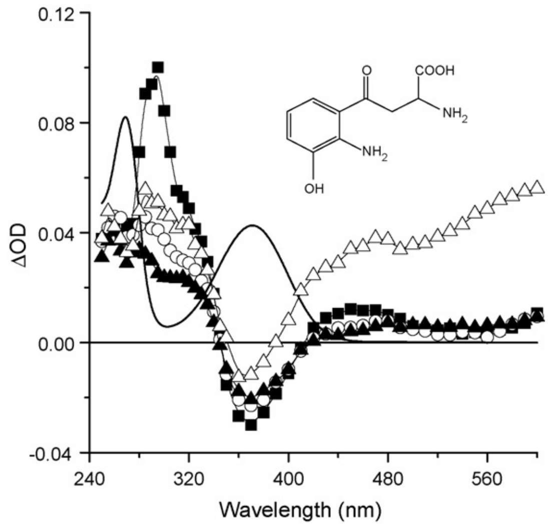

Fig. 2. Transient absorption spectra, obtained in 355 nm photolysis of 2.6×10^{-4} M 3OHKN – open triangles: under Ar, 50 ns after the laser pulse; solid squares: under Ar, 1 μs after the laser pulse; open circles: under Ar in the presence of 5×10^{-3} M acetone, 1 μs after the laser pulse; solid triangles: under O_2 in the presence of 5×10^{-3} M acetone, 1 μs after the laser pulse. Solid line shows the absorption spectrum of 3OHKN.

The comparison of the spectra, obtained 1 μs after the photolysis of KN in neutral and basic solutions (Fig. 3), shows that in neutral solution the electron adduct $KN^{\bullet -}$ undergoes proto-

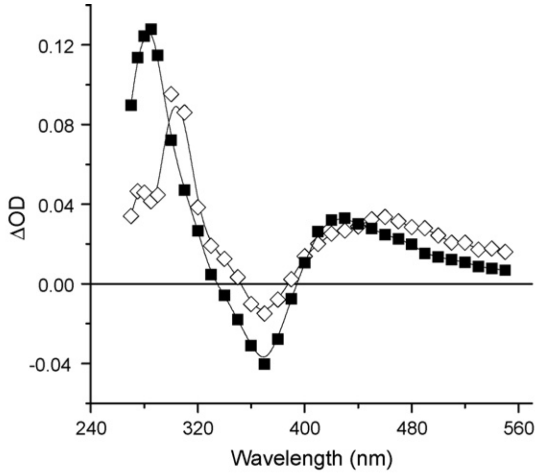

Fig. 3. Transient absorption spectra, obtained 1 μs after 355 nm photolysis of 2×10^{-4} M KN–solid squares: pH 7.0; open diamonds: pH 11.9.

nation yielding the neutral radical $KNH^\bullet$. Thus, the transient absorption spectrum (b), obtained 1 μs after the laser irradiation of Ar-saturated solutions of KN at pH 7.0 (Fig. 1, squares), is a superposition of spectra of three intermediates: cation radical $KN^{\bullet+}$ appears in the course of ionization; electron adduct $KNH^\bullet$ is formed due to electron addition to the ground state KN; the spectrum also includes the contribution from the triplet state ^{T}KN, which is formed under UV irradiation with a quantum yield of approximately 2% [23]. The negative absorption in the 340–390 nm region corresponds to the depletion of the starting material, KN, the optical spectrum of which is shown in Fig. 1 by a solid line.

The spectrum (c), shown in Fig. 1 by circles, was obtained in the presence of 5 mM of acetone in argon-saturated solution. Acetone scavenges solvated electrons with the rate constant $k_{sc} = 6 \times 10^9\ M^{-1}\ s^{-1}$ [33]. The formed electron adduct $Ac^{\bullet-}$ rapidly protonates yielding $AcH^\bullet$. Radical $AcH^\bullet$ is a weak chromophore as compared to KN and its derivatives. Thus, the presented spectrum includes the contributions from two species, ^{T}KN and $KN^{\bullet+}$.

The last spectrum (d) in Fig. 1 (solid triangles) was obtained in the presence of 5 mM of acetone in O_2-saturated solution. Oxygen quenches the triplet state of KN with the rate constant $k_q = 2.1 \times 10^9\ M^{-1}\ s^{-1}$ [23]. Thus, this spectrum corresponds to the absorption of $KN^{\bullet+}$ only.

The presented data allow for the obtaining of the individual spectra of all three intermediates, ^{T}KN, $KNH^\bullet$ and $KN^{\bullet+}$, formed in the photolysis of KN. They are shown in Fig. 4. Solid triangles represent the spectrum of $KN^{\bullet+}$, it was obtained by the correction of spectrum (d) from Fig. 1 for the depletion of the starting compound. The spectrum of the triplet state, ^{T}KN (squares), is the difference between spectra (c) and (d) in Fig. 1, while the spectrum of electron adduct $KNH^\bullet$ (circles) is the difference between spectra (b) and (c). Here, the correction for the starting KN depletion was also made.

The values of the absorption coefficients of intermediates were also extracted from the spectra presented in Fig. 1. At any given wavelength the observed transient absorption can be expressed as

$$\frac{\Delta OD_b}{L} = \varepsilon_{KN^+}C_R + \varepsilon_{KNH}C_R + \varepsilon_T C_T - \varepsilon_{KN}(2C_R + C_T),$$

$$\frac{\Delta OD_c}{L} = \varepsilon_{KN^+}C_R + \varepsilon_T C_T - \varepsilon_{KN}(C_R + C_T),$$

$$\frac{\Delta OD_d}{L} = \varepsilon_{KN^+}C_R - \varepsilon_{KN}C_R$$

Here, ΔOD_b, ΔOD_c and ΔOD_d are the values of the transient absorption in the spectra (b)–(d), correspondingly. L is the optical length, $L = 0.8$ cm. ε_{KN^+}, ε_{KNH}, ε_T, and ε_{KN} are the absorption coefficients of cation $KN^{\bullet+}$, electron adduct $KNH^\bullet$, triplet ^{T}KN, and ground-state KN. C_R and C_T are the concentrations of radicals and triplets, correspondingly ($C_R = C_{KN+} = C_{KNH}$). As one can see from Fig. 1, there is an isobestic point at 350 nm: $\Delta OD_c(350) = \Delta OD_d(350) = 0$. Therefore, $\varepsilon_{KN+}(350) = \varepsilon_T(350) = \varepsilon_{KN}(350) = 4.1 \times 10^3\ M^{-1}\ cm^{-1}$. These values were used to scale the absorption coefficients of $KN^{\bullet+}$ and ^{T}KN for the whole wavelength range. Finally, the absorption coefficient of $KNH^\bullet$, ε_{KNH}, can be easily calculated at wavelengths longer than 420 nm, where the starting compound has no absorption, and the obtained data can be used as a scaling factor for shorter wavelengths. Thus, the spectra in Fig. 4 are presented in absolute values, and the main spectral features of KN and its short-lived intermediates are collected in Table 1. Earlier, the absorption coefficient of the triplet kynurenine at 430 nm was measured by acetone-sensitized photolysis: $\varepsilon_T(430) = 3700\ M^{-1}\ cm^{-1}$ [23], which is in a perfect agreement with the present results.

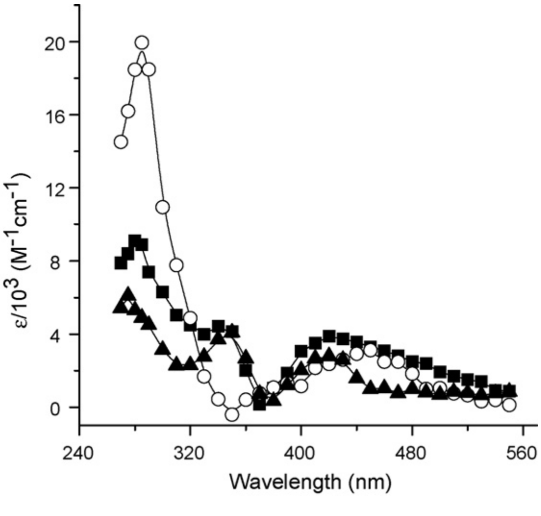

Fig. 4. Absorption spectra of intermediates formed during KN photolysis at pH 7.0 – open circles: protonated electron adduct $KNH^\bullet$; solid squares: triplet ^{T}KN; solid triangles: cation $KN^{\bullet+}$.

Table 1
Spectral features of KN, 3OHKN, and their short-lived intermediates

Substance	State	λ_{max} (nm)	ε_{max} ($M^{-1}\ cm^{-1}$)
Kynurenine	Ground state KN	225	22, 000
		260	7,200
		360	4,500
	Cation radical $KN^{\bullet+}$	275	6, 100
		350	4,100
		420	2,800
	Electron adduct $KNH^\bullet$	285	20, 000
		450	3,100
	Triplet state ^{T}KN	280	9, 100
		340	4,430
		430	3,700
3-OH-Kynurenine	Ground state 3OHKN	230	19, 000
		270	8,000
		370	4,300
	Cation radical $3OHKN^{\bullet+}$	260	21, 200
		315	8,000
	Electron adduct $3OHKNH^\bullet$	295	17, 600
		450	2,000
	Triplet state $^{T}3OHKN$	265	–
		295	–

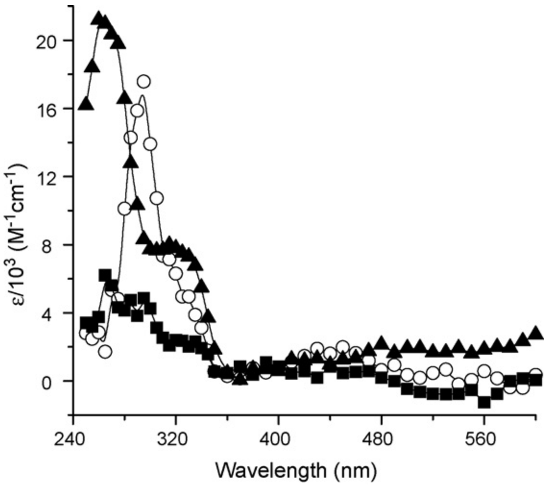

Fig. 5. Absorption spectra of intermediates formed during 3OHKN photolysis at pH 7.0 – open circles: protonated electron adduct 3OHKNH$^•$; solid squares: triplet ^{T}3OHKN (in arbitrary units); solid triangles: cation 3OHKN$^{•+}$.

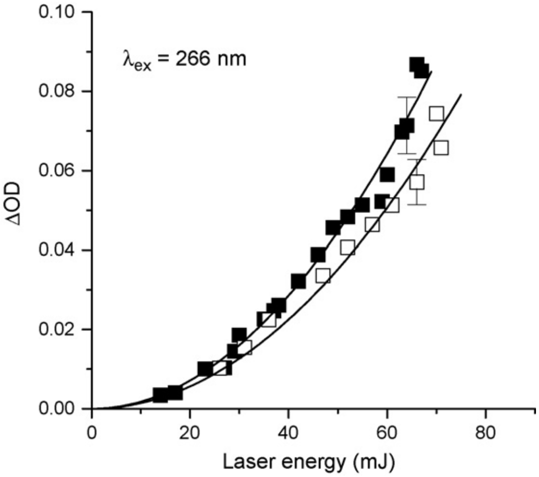

Fig. 7. Dependence of transient absorption measured at 630 nm immediately after the 266 nm laser irradiation of KN (solid squares) and 3OHKN (open squares) solutions on the intensity of laser irradiation.

Similar manipulations were performed with the spectra obtained in 3OHKN photolysis. The individual spectra of electron adduct 3OHKNH$^•$, cation radical 3OHKN$^{•+}$ and triplet state ^{T}3OHKN are shown in Fig. 5, and the spectral features are listed in Table 1. The absorption of the triplet state was very small at all wavelengths above 320 nm, so we were not able to calculate the absolute values of the absorption coefficient of this intermediate. Therefore, its spectrum in Fig. 5 is shown in arbitrary units.

For a better understanding of the mechanism of KN and 3OHKN photoionization, we performed the measurements of the ionization quantum yield at different laser energies for two excitation wavelengths–355 nm and 266 nm. Fig. 6 shows the dependences of transient absorption measured at 630 nm immediately after the 355 nm laser irradiation of 2.07×10^{-4} M solution of KN and 2.48×10^{-4} M solution of 3OHKN on the output laser energy. Fig. 7 shows similar dependences, obtained with the laser excitation at 266 nm. All measurements were performed at room temperature in neutral (pH 7.2) solutions. Apparently, the yield of the solvated electron formation depends on the laser power quadratically rather than linearly. The second-degree functions are shown in Figs. 6 and 7 by solid lines as a visual guide. The photoionization quantum yields were calculated using the absorption coefficient of solvated electron at 630 nm $\varepsilon_e(630) = 1.5 \times 10^4\ \mathrm{M}^{-1}\ \mathrm{cm}^{-1}$ [34], the dependences of the photoionization quantum yield on the laser energy are presented in Fig. 8. All four dependences show a good linear fit, testifying that at both excitation wavelengths 355 and 266 nm the photoionization of KN and 3OHKN proceeds by a biphotonic mechanism.

A precursor for the biphotonic ionization is most likely the triplet state. It has been reported that 3OHKN demonstrates very short fluorescence time–32 ps [21]. Thus, the lifetime of the singlet excited state is too short to absorb a second photon within

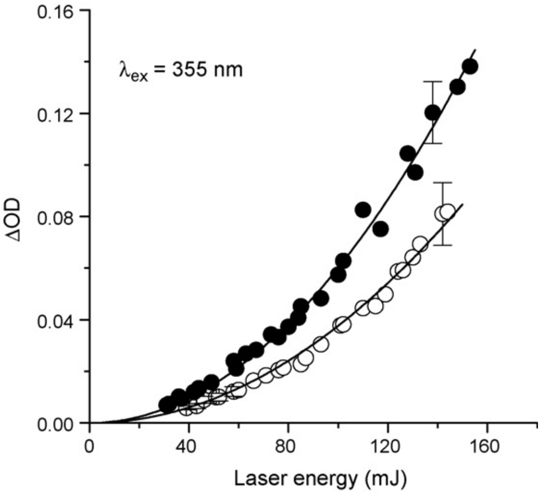

Fig. 6. Dependence of transient absorption measured at 630 nm immediately after the 355 nm laser irradiation of KN (solid circles) and 3OHKN (open circles) solutions on the intensity of laser irradiation.

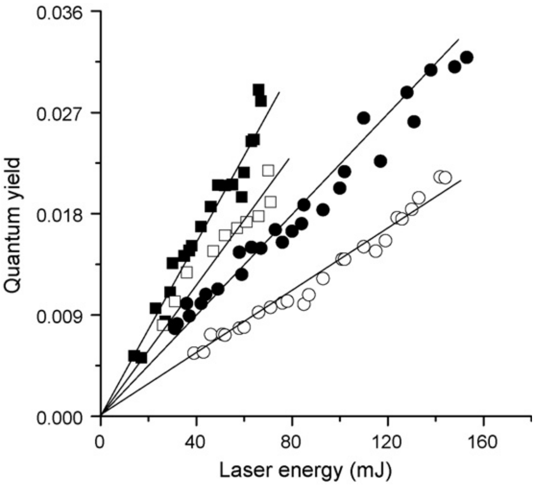

Fig. 8. Dependence of photoionization quantum yield on the intensity of laser irradiation – solid squares: KN, 266 nm photolysis; open squares: 3OHKN, 266 nm photolysis; solid circles: KN, 355 nm photolysis; open circles: 3OHKN, 355 nm photolysis.

the duration of the same laser pulse (about 7 ns). The results of the present work also speak in favor of a triplet precursor for ionization. Indeed, both ^{T}KN and ^{T}3OHKN have stronger absorption at 266 nm than at 355 nm (see Figs. 4 and 5), and so the photoionization quantum yield at 266 nm is higher (Fig. 8). The signal of ^{T}KN at all wavelengths is stronger than that of ^{T}3OHKN, which means that either triplet yield in the KN photolysis is higher, or its absorption is stronger. Correspondingly, KN demonstrates higher yields of photoionization than 3OHKN.

Thus, the common reaction scheme of KN and 3OHKN photolysis can be presented as following:

$$\mathrm{M} \xrightarrow{h\nu} {}^{\mathrm{S}}\mathrm{M}^{*} \xrightarrow{\mathrm{ISC}} {}^{\mathrm{T}}\mathrm{M}, \qquad {}^{\mathrm{T}}\mathrm{M} \xrightarrow{h\nu} \mathrm{M}^{\bullet +} + \mathrm{e}^{-},$$

$$\mathrm{M} + \mathrm{e}^{-} \xrightarrow{k_{\mathrm{e}}} \mathrm{M}^{\bullet -}, \qquad \mathrm{M}^{\bullet -} + \mathrm{H}^{+} \rightleftarrows \mathrm{MH}^{\bullet}$$

In conclusion, our study shows that the triplet state is the key intermediate in the photolysis of KN and its derivatives. Under intensive laser irradiation, the triplet state can absorb a second light quantum and undergo photoionization. This reaction results in the formation of cation radical $\mathrm{M}^{\bullet +}$ and electron adduct $\mathrm{M}^{\bullet -}$, the latter at neutral pH converts into neutral radical $\mathrm{MH}^{\bullet}$. The same radicals can also appear in the reactions of KN triplet state with electron donors and acceptors, so their spectral features reported here could be important for understanding reaction mechanisms. The reactivity of the kynurenine triplet state toward amino acids and other biologically important molecules is currently under study in our lab and will be published in a separate paper.

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