Water concentration dependent photochemistry of ketoprofen in aqueous solutions[†]

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Received 16th September 2009, Accepted 9th February 2010 First published as an Advance Article on the web 16th March 2010 DOI: 10.1039/b919330h

Ketoprofen is an important photosensitive drug molecule that has received much attention for the study of its photochemistry in different solvents. In this paper, nanosecond time-resolved resonance Raman spectroscopy was utilized to investigate the photochemistry of ketoprofen in aqueous solutions with varying water concentrations. The rate constants and reaction mechanism of ketoprofen are strongly dependent on the concentration of the solvent. In neat acetonitrile and solvents with low concentrations of water (like water–acetonitrile $\leq 1:1$, v/v), ketoprofen exhibits mostly benzophenone-like photochemistry to generate a triplet state which in turn produces a ketyl radical-like species by a hydrogen abstraction reaction. However, in solvents with very high concentrations of water (such as water–acetonitrile $\geq 9:1$, v/v), the triplet state ketoprofen is observed first and then undergoes a prompt decarboxylation process to form a triplet protonated biradical carbanion species. For solvents with moderate higher water concentrations (such as between 50% and 90% water by volume), the hydrogen abstraction and decarboxylation processes are two competitive pathways with different rate constants. The triplet state of ketoprofen will simultaneously produce a ketyl radical species and a triplet protonated biradical carbanion species with the amount of each species dependent on the water concentration.

Introduction

Ketoprofen (KP) is a common nonsterioidal anti-inflammatory drug (NSAID) that is widely used for moderate acute and chronic painful conditions to improve the tolerability profile of NSAIDs. However, KP can induce photosensitivity and phototoxicity due to its specific structure and electronic features.^{1,2} A large number of studies have been performed in recent years on both the phototoxic and photoallergic reactions induced by KP so as to estimate the safety of KP and also to investigate the mechanism(s) of the photosensitivity reactions when KP is exposed to UV light.^{3,4} These results show that the photochemistry of KP in aqueous solution is strongly influenced by its acid-base chemistry.⁵ The neutral form of ketoprofen that exists in the absence of a base exhibits typical benzophenone-like photochemistry,5,6 while the deprotonated ketoprofen that exists in the presence of a base undergoes an efficient photodecarboxylation process.⁷ A number of investigations have been done to attempt to better elucidate the mechanism(s) that cause KP's phototoxicity and to explore the acid-base influence on the photochemistry of KP in aqueous solution.⁸⁻¹¹ KP was studied in water and in phosphate buffer solutions in the 5.7-11.0 pH range by laserinduced optoacoustic spectroscopy¹² and these studies showed that the decarboxylation from the excited singlet deprotonated ketoprofen did not depend on pH or on the presence of a

buffer, while the decay of the carbanion in the nanosecond time-scale displayed a clear pH-dependence. The main reason this affected the characteristic of the carbanion is that after the decay of the carbanion it will form a long-lived species (triplet protonated biradical carbanion species) at low pH values and finally lead to the direct formation of the final product (3-ethylbenzophenone) at higher pH values. Recently, nanosecond time-resolved resonance Raman (ns-TR³) and density functional theory (DFT) calculations were used to investigate the photochemistry of (S)-ketoprofen in different solvents.⁶ In aqueous solution, KP exhibits a pH dependent photochemistry in a 1:1 water-acetonitrile (volume ratio) solution. In acidic aqueous and neutral solution, the triplet state ketoprofen (³[KP]) is formed through intersystem crossing (ISC) from the first excited singlet state (S_1) , and then the ³[KP] species produces an arylphenylketyl (ArPK) radical via a hydrogen abstraction reaction with the surrounding water molecules. In contrast, a prompt decarboxylation process will take place from the triplet state deprotonated ketoprofen $({}^{3}[KP^{-1}])$ and then involves the formation of the triplet protonated biradical carbanion species in a 1:1 water-acetonitrile alkaline and 7:3 PBS-acetonitrile aqueous solutions.⁶ Therefore the photochemistry of KP appears determined by the precursors, the neutral form of ${}^{3}[KP]$ or the deprotonated ${}^{3}[KP^{-1}]$.

In an attempt to establish the nature of the carbanion's precursor, Scaiano and coworkers have conducted studies on the photochemistry of KP using transient absorption and quenching experiments in organic solvent–water mixtures.⁵ They proposed that the same rate constant for the decay of transient species and spectral characteristics were obtained in various water–acetonitrile mixtures in the absence of base.

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 $[\]dagger$ Electronic supplementary information (ESI) available: Raman spectra; nS-TR 3 spectra. See DOI: 10.1039/b919330h

However, the rate constant of the decay of the carbanion showed an interesting dependence on the mole fraction of water.⁵ This result appeared to be consistent with later ns-TR³ experiments that showed the triplet protonated biradical carbanion decays faster in a 0.5 mM NaOH aqueous solution than in a PBS solution.⁶

It is difficult to clearly distinguish various intermediates that have similar electronic absorption spectra and time-scales in time-resolved transient absorption experiments due to the spectral overlap of the absorption spectra on similar time scales for the different transient species that may be present.^{13–17} In these cases it is useful to employ time-resolved vibrational spectroscopy techniques that can more clearly distinguish the various transient species present by their vibrational frequencies that contain structural information and can serve as a fingerprint for each species. In this work, we have used timeresolved resonance Raman (TR³) spectroscopy, a type of time-resolved vibrational spectroscopic technique, which is a powerful tool to obtain valuable information about the identity, structure and properties of chemical reaction intermediates.¹⁸⁻²⁰ We have recently utilized ns-TR³ spectroscopy to investigate the photochemistry of benzophenone in aqueous solutions and KP in different solvents^{6,13} and these studies provided new insight into the identity, structure and properties of several intermediates associated with the hydrogen abstraction and decarboxylation reactions observed in these two molecular systems.

In this article, the photochemistry of KP in aqueous solutions with varying water-acetonitrile ratios were studied using nanosecond time-resolved resonance Raman spectroscopy (ns-TR³). The ns-TR³ spectra provide vibrational spectroscopic information that can be used to help elucidate the photochemical mechanism(s) and solvent effects on the intermediates involved in the reactions. The experimental results indicate that the reaction pathways for KP are strongly dependent on the solvent ratio of water and acetonitrile in the solvent system. In aqueous solutions with less than 50% water, the ³[KP] is mainly observed and then forms an arylphenylketyl (ArPK) radical via a hydrogen abstraction reaction with water. However, in aqueous solutions with 90% or greater water, the triplet state mostly promptly experiences a decarboxylation process to form a triplet biradical carbanion species. Both hydrogen abstraction and decarboxylation occur and take place to an appreciable extent in aqueous solutions with water concentrations between 50% and 90% water.

Experimental and computational methods

(S)-Ketoprofen (KP) was obtained from Aldrich with 99% purity and used as received. Spectroscopic grade acetonitrile (MeCN), potassium dihydrogen phosphate, anhydrous sodium phosphate dibasic of reagent grade from Aldrich, and deionized water were used for preparing sample solutions. Experimental sample solutions of KP with a concentration of 1 mM in neat MeCN and mixed aqueous solvents were used in the ns-TR³ experiments. Seven sample solutions including neat MeCN, 1:9 H₂O–MeCN (10% water), 3:7 H₂O–MeCN (30% water), 1:1 H₂O–MeCN (50% water), 7:3 H₂O–MeCN (70% water), 9:1 H₂O–MeCN (90% water) and 9:1

PBS-MeCN (90% PBS) were used as solvents and unless specified, all of the mixed solvent ratios are of volume ratio. PBS was prepared by mixing 2.314 g KH₂PO₄, 7.384 g Na_2HPO_4 in a 1000 ml solution with a final pH value of 7.4. The ns-TR³ experiments were done using an experimental apparatus and methods discussed in detail previously,^{21,22} and only a brief description will be given here. The 266 nm pump laser pulse generated from the fourth harmonic of a Nd:YAG nanosecond pulsed laser and a 319.9 nm probe laser pulse generated from the third anti-Stokes hydrogen Raman shift laser line from the second harmonic were employed in the ns-TR³ experiments. The energy for the pump and probe pulses were in the range of 2.5-3.5 mJ with a 10 Hz repetition rate. The two Nd:YAG lasers were synchronized electronically by a pulse delay generator to control the time delay of the pump and probe lasers with the time delay between the laser pulses monitored by a fast photodiode and 500 MHz oscilloscope. The time resolution for the ns-TR³ experiments was approximately 10 ns. The pump and probe laser beams were lightly focused onto the flowing sampling system and the Raman light was collected using reflective optics into a spectrometer whose grating dispersed the light onto a liquid nitrogen cooled CCD detector. The Raman signal was accumulated for 30 s by the CCD before reading out to an interfaced PC computer and 10-20 scans of the signal were added together to get a resonance Raman spectrum. The ns-TR³ spectra presented here were obtained by the subtraction of a resonance Raman spectrum with negative time delay of -100 ns (probe-before-pump spectrum) from the resonance Raman spectrum with a positive time delay (pump-probe spectrum) and the Raman shifts were calibrated by the known MeCN solvent's Raman bands with an estimated accuracy of 5 cm^{-1} . A Lorentzian function was applied to integrate the relevant Raman bands to determine the decay and growth kinetics of the species observed in the experiments (details for how the kinetics of the intermediate was obtained are shown in the ESI[†]).

Solvent effects were considered by UB3LYP/6-311G** optimizations of the gas-phase stationary points using a simple SCRF method based on a polarizable continuum model (PCM).²³ All of the density functional theory calculations presented in this work made use of the Gaussian 03 program suite²⁴ operated on the High Performance Computing cluster (HPCPOWER) at the University of Hong Kong.

Results and discussion

KP is a benzophenone derivative and hence exhibits benzophenone-like photochemistry in acidic and neutral solution. The triplet state benzophenone (3 [BP]) (without a carboxyl group when compared with KP) will abstract hydrogen from a water molecule in neutral solution (1:1 H₂O–CH₃CN) to form a ketyl radical species, but no ketyl radical intermediate is observed in CH₃CN solution.¹⁷ This indicates that the 3 [BP], and very likely KP as well, do not abstract hydrogen easily from CH₃CN but from a water molecule instead. This may be due to the hydrogen bond attraction in water moves the molecules closer together and forms an electrostatic-type attraction between them. Therefore, the propensity for 3 [BP] and ³[KP] to abstract hydrogen from H₂O over CH₃CN (the latter having an apparently weaker C-H bond) may be due to the initial hydrogen bonding of the O-H to the carbonyl of the benzophenone moiety. This might be sufficient to reduce the activation barrier such that abstraction occurs *via* a pathway that seems intrinsically less likely based on examination of the thermochemisty (that is, the relative bond strengths) that does not consider explicit hydrogen bonding effects on the reaction. Besides, the evidence for hydrogen abstraction from water by a triplet of benzophenone was previously found in water solution.²⁵ The initial very fast photophysical processes produce the triplet state ketoprofen (³[KP]) that can react with a nearby molecule to form a ArPK radical via a hydrogen abstraction reaction in a 1:1 H₂O-MeCN neutral solution and also in an acidic solution and this reaction has been observed by ns-TR³ experiments employing a 266 nm pump laser and 319.9 nm probe laser excitation wavelengths.⁶ The growth time constant of the ArPK radical was determined to be 100 ns in a 1:1 H₂O-MeCN solution and 204 ns in a 1:1 D₂O-MeCN solution from ns-TR³ experiments. A typical primary kinetics isotope effect of $k_D/k_H = 2$ was observed.⁶ This demonstrates that the ³[KP] species reacts with the poor hydrogen donor water molecules by a hydrogen abstraction process to form the ketyl radical species. In order to learn more about how water molecules are involved in the photochemistry of KP, ns-TR³ experiments were carried out in H₂O-MeCN solutions containing 10%, 30%, 50%, 70%, 90% (vol%) water.

Triplet state hydrogen abstraction—generation of the ArPK radical

Fig. 1 shows ns-TR³ spectra of 1 mM KP obtained in a 10% water-MeCN solution. Two intermediates can be observed over the 10 ns to 1 ms time-scale. The first intermediate has characteristic Raman bands at 966, 1173, 1121 and 1538 cm⁻¹ and is assigned to the ³[KP] species because the resonance Raman spectrum of this species displays excellent agreement with that of an authentic ³[KP] spectrum which was obtained in neat acetonitrile solvent (the comparison of these ns-TR³ spectra are shown in Fig. 2).6 The integrated areas of the characteristic Raman band at 1538 cm⁻¹ of the ³[KP] species as a function of the time delay can be fit satisfactorily by a single exponential function and gave a time constant for the decay of *ca*. 119 ns (see Fig. 1S[†]). As the ³[KP] decays, a small shoulder peak (at 1582 cm⁻¹) belonging to the second species gradually increases and it appears that the ³[KP] is the precursor of the second intermediate species. The second species is detected at 10 ns time delay, which appears at an earlier time than in neat acetonitrile where the 1582 cm⁻¹ Raman band appears at 30 ns and this indirectly suggests that water molecules are involved in the decay of the ³[KP] intermediate.

Fig. 3 shows the ns-TR³ spectra of 1 mM KP obtained in 30% and 50% water–MeCN solutions and these spectra are very similar to those shown in Fig. 1 except for the kinetics of the transient species. The ³[KP] intermediate is observed in the range of the time resolution of the ns-TR³ experiments and the Raman band (1582 cm⁻¹) for the second species is also



Fig. 1 Ns-TR³ spectra of 1 mM KP in 1:9 H_2O –MeCN solution obtained using a 266 nm pump excitation wavelength and a 319.9 nm probe wavelength at various time delays indicated next to the spectra. The asterisk (*) marks regions affected by solvent subtraction artifacts and/or stray light.



Fig. 2 Comparison of the experimental resonance Raman spectrum of KP in a 1:9 H₂O–MeCN solution acquired at a time delay of 5 ns (top) and the authentic ³[KP] spectrum in neat acetonitrile solvent acquired at a time delay of 5 ns (bottom). The asterisk (*) marks regions affected by solvent subtraction artifacts and/or stray light.

observed at 10 ns time delay. The intensity of the second species gradually becomes larger as the concentration of water increases. The ns-TR³ spectra obtained at time delays later than 400 ns belong to the second species and the resonance Raman spectrum of the second species in 30% and 50% aqueous solution is almost identical to that of an authentic ArPK radical which is obtained in neat isopropanol solvent and detected with the same probe wavelength as used here.⁶ Hence the second species observed on the nanosecond time

scale in 30% and 50% water solutions shown in Fig. 3 is reasonably assigned to the ArPK radical. Although the ArPK radical is typically generated in H-donor solvents such as alcohols and amines, the observation of the ArPK radical here in 10%, 30% and 50% water–MeCN solutions is consistent with previous results that reported the observation of the ketyl radical species.^{6,13} This observation provides valuable evidence for a hydrogen abstraction reaction with water molecules by the ³[KP] intermediate.

In order to determine the kinetics of those intermediates, the contribution of other species generated at post time delays need to be removed. The characteristic 1538 and 1582 cm⁻¹ Raman bands in Fig. 3 which belong to the ³[KP] and ArPK radical respectively were fit with a Lorentzian function to obtain the time dependence of these two species and the kinetics determined from this analysis are displayed in Fig. 3. In the 30% water–MeCN solution, the time dependence of the 1538 cm⁻¹ Raman band integrated areas for the ³[KP] intermediate were fit by a single-exponential function with a

decay time constant of ca. 107 ns and the time dependence of the 1582 cm⁻¹ Raman band integrated areas for the ArPK radical were fit by a two-exponential function with a ca. 109 ns growth time constant and a ca. 230 ns decay time constant. In the 50% water solution, the time dependence of the 1538 $\rm cm^{-1}$ Raman band integrated areas for the ³[KP] intermediate were fit by a single-exponential function with a decay time constant of *ca*. 97 ns and the time dependence of the 1582 cm^{-1} Raman band areas for the ArPK radical were fit by a two-exponential function with a ca. 118 ns growth time constant and a ca. 910 ns decay time constant. Examination of the ns-TR³ spectra in the 10%, 30%, 50% water-MeCN solutions shows that the time constant for the decay of the ³[KP] intermediate gradually decreases as the water concentration increases and this suggests that the reaction rate of the hydrogen abstraction reaction for the ³[KP] species is significantly affected by the surrounding water molecules. The time constant for the growth of the ArPK radical is somewhat longer than the time constant for the decay of the ³[KP] intermediate suggests that



Fig. 3 Ns-TR³ spectra of 1 mM KP in 3:7 (A, left) and 1:1 (B, right) H₂O–MeCN solution obtained using 266 nm pump excitation wavelength and 319.9 nm probe wavelength at various time delays indicated next to the spectra and below are its corresponding kinetics. The asterisk (*) marks regions affected by solvent subtraction artifacts and/or stray light. In 30% water solution, the time constant for the decay of the ³[KP] (squares) is *ca.* 107 ns and the time constant for the growth and decay of the ArPK radical (triangles) are *ca.* 109 ns and *ca.* 230 ns respectively. In 50% water solution, the time constant for the decay of the ³[KP] (squares) is *ca.* 97 ns and the time constants for the growth and decay of the ArPK radical (triangles) are *ca.* 118 ns and *ca.* 970 ns respectively. The inset displays an expanded view of the kinetics for the early 200 ns time delay.

a small portion of the 3 [KP] species may be quenched by oxygen under the open air conditions of the experiments. The time constant for the decay of the ArPK radical gradually increases as the water concentration increases. This seems mainly attributed to there being more ArPK radicals generated as the water concentration increases. Therefore, in the 50% water solution, the ArPK radical is still observed at a 1.5 µs time delay.

As the ArPK radical decays, a new species is observed after the decay of the ArPK radical which may form from the self-coupling reaction between ArPK radicals or the crosscoupling reaction between ArPK radicals and water molecules. Different concentrations of KP and pump power conditions experiments were carried out to determine the decay time constants of the ArPK radical. These results make it clear that the kinetics for the decay of ArPK radical does not depend on the concentration of KP and the pump power conditions. This implies that the third species does not arise from the self-coupling reaction of the ArPK radical. On the contrary, it is more likely to couple with the surrounding water molecules which are present in a large excess. Therefore, the third species is tentatively assigned to (2S)-2-[3-(hydroxyl-(4hydroxycyclohexa-2,5-dienylidene)-methyl)-phenyl]-propanoic acid (p-OH).6

Triplet state hydrogen abstraction and triplet state decarboxylation reactions-simultaneously produce the ArPK radical and the triplet protonated biradical carbanion species

The rate constants exhibit a water concentration dependence in solutions with less than 50% water present. In addition, the kinetics of the decarboxylation reaction have been examined due to the strong dependence of the rate constant on the polarity of the solvent.²⁶ The polar protic solvent was proposed to be the donor of a proton or hydrogen to aid the formation of an intramolecular proton transfer as the initial step to induce the photodecarboxylation.²⁷ Thus a further increase in the concentration of water may lead KP to exhibit an obvious decarboxylation process. Fig. 4 displays the ns-TR³ spectra of 1 mM KP obtained in a 7:3 H₂O-MeCN aqueous solution. The Raman bands at 966, 1173, 1121 and 1538 cm⁻¹ can be observed at early time delays and these four Raman bands are assigned to the ³[KP] species because the spectra of this species are essentially identical to that of the ³[KP] intermediate obtained in neat acetonitrile. Examination of the ns-TR³ spectra in Fig. 4 shows that the resonance Raman signals of the intermediates are greatly enhanced and an obvious spectral change is present so that four new bands appear at 966, 1024, 1282 and 1519 cm^{-1} . Fig. 5 shows a comparison of the experimental ns-TR³ spectrum of KP in a 1:1 water-MeCN solution acquired at a time delay of 800 ns (top) and the experimental ns- TR^3 spectrum of KP in a 7:3 water-MeCN solution acquired at a time delay of 800 ns (bottom). Our previous work demonstrated that a triplet protonated biradical carbanion species was produced through a decarboxylation process in a PBS solution when 266 nm is used as the pump laser. Fig. 6 shows a comparison of the experimental resonance Raman spectrum obtained in a 7:3 water-MeCN solution acquired at a time delay of 800 ns and



Fig. 4 Ns-TR³ spectra of 1 mM KP in a 7:3 H₂O–MeCN solution obtained using a 266 nm pump excitation wavelength and a 319.9 nm probe wavelength at various time delays indicated next to the spectra. The asterisk (*) marks regions affected by solvent subtraction artifacts and/or stray light.

the spectrum of the triplet protonated biradical carbanion obtained in a 9:1 PBS-MeCN solvent acquired at a time delay of 800 ns. The Raman characteristic feature at 1519 cm^{-1} of the triplet protonated biradical carbanion species can be seen to emerge in the ns- TR^3 spectra obtained in the 70% neutral aqueous solution demonstrates that this new species is the triplet protonated biradical carbanion species. The overlapping of the resonance Raman spectra for the triplet protonated biradical carbanion and ArPK radical leads to an up-shift of two Raman bands (987 cm⁻¹ and 1177 cm⁻¹). Therefore, two intermediates are clearly produced in the same range of time delays and this is easily observed in the ns-TR³ spectra. Previous time-resolved transient absorption studies had difficulties in clearly distinguishing these intermediates due to their overlapping broad absorption spectra.¹³ However, timeresolved resonance Raman can readily overcome this problem because each intermediate has its own fingerprint vibrational frequencies with higher resolution than their absorption spectra.

In order to remove the contributions from other intermediates (including the 3 [KP] intermediate and other species) generated at post time delays, the Raman spectrum of the ArPK radical was extracted by subtracting the spectrum of the 3 [KP] obtained in neat acetonitrile and the spectrum of the triplet protonated biradical carbanion obtained in the PBS solution from that acquired at a different time delay in the 70% water solution shown in Fig. 4. Fig. 2S† shows the extracted ns-TR³ spectra of the ArPK radical. The characteristic Raman bands at 1538 and 1582 cm⁻¹ in Fig. 4 belonging to the 3 [KP]



Fig. 5 Comparison of the experimental ns-TR³ spectrum of KP in a $1:1 \text{ H}_2\text{O}$ -MeCN solution acquired at a time delay of 800 ns (top) and the experimental ns-TR³ spectrum of KP in a 7:3 H₂O-MeCN solution acquired at a time delay of 800 ns (bottom). The asterisk (*) marks regions affected by solvent subtraction artifacts and/or stray light.



Fig. 6 Comparison of the experimental ns- TR^3 spectrum of KP in a 7:3 H₂O–MeCN solution acquired at a time delay of 800 ns (top) and the spectrum of the triplet protonated biradical carbanion obtained in a 9:1 PBS–MeCN solution acquired at a time delay of 800 ns (bottom). The asterisk (*) marks regions affected by solvent subtraction artifacts and/or stray light.

ArPK radical respectively were fit with a Lorentzian function to obtain the time dependence of these two species and the kinetics obtained from this analysis are displayed in Fig. 7. The integrated areas of the characteristic 1538 cm^{-1} Raman band for the ³[KP] intermediate were fit by a single-exponential function with a time constant of ca. 46 ns. The integrated areas of the characteristic 1582 cm⁻¹ Raman band for the ArPK radical were fit by a two-exponential function with a ca. 220 ns growth time constant and a ca. 1120 ns decay time constant. As mentioned above, the 3 [KP] species is the precursor for the formation of the ArPK radical. The time constant for the decay of the ³[KP] (46 ns) is noticeably smaller than the growth of the ArPK radical (220 ns), within the uncertainty of the measurements, and this indicates that another pathway for the decay of the ³[KP] species exists in the 70% water solution. Although we cannot exclude that there may be other pathways



Fig. 7 The time dependence of the 1538 cm⁻¹ Raman band integrated areas of 3 [KP] (closed squares) in a 7:3 water–MeCN solution were fit by a single-exponential function with a decay time constant of *ca*. 46 ns and the time dependence of the 1582 cm⁻¹ Raman band areas of the ArPK radical (closed triangles) were fit by a two-exponential function with a *ca*. 220 ns growth time constant and a *ca*. 1120 ns decay time constant. The inset displays an expanded view of the kinetics for the 3 [KP] intermediate. The data displayed here were derived from the ns-TR³ spectra shown in Fig. 4.

giving rise to the triplet protonated biradical carbanion at higher water concentrations, it is possible that the ³[KP] species decays and then generates the triplet protonated biradical carbanion.

There appear to be three pathways for the decay of the ³[KP] intermediate: one is quenching by oxygen dissolved in the water solution with a quenched rate constant k_1 ; the second is the formation of the ArPK radical by a hydrogen abstraction reaction with water molecules with a reaction rate constant k_2 (220 ns⁻¹); the third produces the triplet protonated biradical carbanion species through a decarboxylation process with a reaction rate constant k_3 . The observed rate constant (46 ns⁻¹) for the decay of the ³[KP] in the 70% water solution is the sum of these three reaction pathways. Therefore, the growth time constant for the ${}^{3}[KP]$ photodegradation by the decarboxylation reaction in the 70% water solution is ca. 102 ns (obtained using 46 ns⁻¹ = 220 ns⁻¹ + 136 ns⁻¹ + k_2). Oxygen will quench the 3 [KP] with a *ca*. 136 ns time constant in neat acetonitrile. Since the solubility of oxygen in the 70% water solution is smaller than that of in neat acetonitrile, the quenching rate k_1 should be smaller than 136 ns⁻¹. Thus, the reaction rate constant k_2 for the decarboxylation process is likely larger than 102 ns⁻¹. In other words, the growth time constant for the ³[KP] photodegradation by the decarboxylation reaction in the 70% water solution is smaller than 102 ns.

It was difficult to obtain the decay time constant for the triplet protonated biradical carbanion due to overlapping of the resonance Raman spectra. The observation of the triplet protonated biradical carbanion in the 70% water solution indicates the decarboxylation process becomes more favored at higher concentrations of water in aqueous solutions. The ns-TR³ spectra obtained in 90% water solution are similar to those obtained in 70% water solution and also to those obtained in 90% PBS solution (shown in Fig. 3S). Examination of Fig. 8



Fig. 8 Ns-TR³ spectra of 1 mM KP in a 9:1 H₂O–MeCN solution obtained using a 266 nm pump excitation wavelength and a 319.9 nm probe wavelength at various time delays indicated next to the spectra are shown. The asterisk (*) marks regions affected by solvent subtraction artifacts and/or stray light.

shows that the Raman band of 1519 cm^{-1} is obviously enhanced compared with the one obtained in 70% water solution at the same time delay. This indicates that a noticeable amount of ³[KP] will experience the decarboxylation to generate the triplet protonated biradical carbanion.

An interesting phenomena observed in our study is that the decay time constants of the triplet protonated biradical carbanion in the 90% PBS solution (1770 ns, shown in Fig. 4S[†]) and the 90% water solution (1547 ns, shown in Fig. 9) are noticeably longer than the decay time constant of triplet protonated biradical carbanion (411 ns) in the 70% PBS solution.⁶ This indicates that increasing the concentration of water can help KP undergo the decarboxylation process to generate the carbanion species more easily. On the other hand, this also provides an implication that water can stabilize the triplet protonated biradical carbanion but the reason for this stabilization is not clear. Thus the solvent effects on the carbanion and triplet protonated biradical carbanion were investigated using the UB3LYP/6-311G** optimized geometries of the gas-phase stationary points and then using the SCRF method based on a polarizable continuum model (PCM) developed by Tomasi and co-workers.²³ The calculations were done for acetonitrile and water solvents and show that water can noticeably decrease the free energy of the carbanion and triplet protonated biradical carbanion by about *ca*. 1.3 and *ca*. 0.6 kcal respectively and this suggests that the solvent polarity may have a noticeable impact on the lifetime of the triplet protonated biradical carbanion. It is important to note that



Fig. 9 The time dependence of the 1578 cm⁻¹ Raman band integrated areas of the triplet protonated biradical carbanion species were fit by a two-exponential function with a *ca*. 1547 ns decay time constant in 9:1 H₂O–MeCN solution (closed squares).

most biological reactions take place in water solution environments and our results suggest that the triplet protonated biradical carbanion is very likely responsible for much of the photosensitization and phototoxicity of KP. Scaiano and coworkers tried to extend the life-time of the carbanion to explore the carbanion's reactivity and our results appear consistent with theirs.²⁸

Proposed photochemical mechanism of KP in aqueous solutions with varying concentrations of water

The propionic acid chain substitution in KP results in a ground state acid–base equilibrium with a $pK_a = 4.7$ and this indicates that the main component in a neutral aqueous solution is the neutral KP.²⁹ Thus, the neutral KP is excited and undergoes efficient photophysical processes to produce the ³[KP] which appears to be a predominant intermediate formed in a neutral aqueous solution. According to our experimental results, the ³[KP] species exhibits a water concentration dependent photochemistry. Scheme 1 shows a proposed photochemical mechanism for KP in water solutions with varying concentrations of water. In water solutions with lower water concentrations (like those with less than 50% water concentration), the photolysis of KP leads to formation of the first excited singlet state (S_1) and that then forms the ³[KP] species through a highly efficient intersystem crossing (ISC) process.^{6,30} This triplet state easily undergoes mainly photoreduction by hydrogen abstraction from water molecules and forms the ArPK radical with different rate constants depending on the water concentration in the aqueous solutions. For aqueous solutions with a modest to high water concentration (like solutions with between 50% and 90% water), the 3 [KP] species appears to lead to appreciable degrees of hydrogen abstraction and decarboxylation reactions. These two photochemical pathways lead to formation of the ArPK radical and the triplet protonated biradical carbanion with different kinetics dependent on the water concentration of the solution. A further increase in the water concentration (such as aqueous solutions with 90% and greater concentration of water) leads to the decarboxylation process becoming more predominant

to generate a greater degree of the carbanion species. The detailed mechanism for the decarboxylation of the neutral triplet state KP is not completely clear and further investigation on this mechanism will be done in the future. Finally, we note that the triplet protonated biradical carbanion can undergo a water-assisted proton shift and radiationless decay to the singlet ground state 3-ethylbenzophenone product.^{12,31} As mentioned above, KP is a weak acid, the dissociated carboxylate form should be in equilibrium with the acid form for either the ground state KP or triplet state KP. A small amount of deprotonated KP will easily undergo decarboxylation and give arise to the carbanion intermediate (shown in Scheme 1). Scaiano and coworkers reported that the strong transient absorption peak of the carbanion intermediate appeared at 650 nm.^{5,7} In the case of the ns-TR³ experiments performed on KP reported here, the carbanion intermediate may not have been observed when 319.9 nm was used as the probe laser wavelength in the experiments. One of our recent papers⁶ reported TD-DFT calculations for the carbanion and triplet protonated biradical carbanion and found that the triplet protonated biradical carbanion has a higher oscillator strength near the probe laser wavelength (319.9 nm) while the absorption spectrum of the carbanion displayed a relatively weak oscillator strength near the 319.9 nm probe laser wavelength. Thus, the carbanion intermediate was probably not observed in ns-TR³ experiments because it had a very small Raman signal due to its weak resonance enhancement. The carbanion and its resonant structure will promptly receive a proton and generate the triplet protonated biradical carbanion species in aqueous solution.⁶ The triplet protonated biradical carbanion transforms into the final product (3-ethylbenzophenone) presumably through electron transfer.12

The photochemistry of KP appears to be strongly influenced by its molecular environment.^{32,33} When dissolved in a plasma which is similar to a water predominant environment (like the 90% water solution examined here), our results suggest that the photodecarboxylation should be the predominant process and the KP triplet state should be short-lived. In contrast, when KP is immersed in a protein hydrophobic core which would be more like an acetonitrile solution with lower concentrations of water (such as the neat MeCN and 30% water–MeCN solutions examined here), a higher contribution of the hydrogen abstraction would be expected. Our current study indicates that the photochemistry of KP has substantial water concentration dependence and suggests that the ratio or concentrations of the ArPK radical and the triplet protonated biradical carbanion reactive intermediates will vary with the hydrophilic *versus* hydrophobic nature of the micro-environments present in the different parts of complex biological systems.

Conclusions

We have explored the photochemistry of KP in aqueous solutions with varying water concentrations using nanosecond time-resolved resonance Raman spectroscopy. The ns-TR³ spectra reveal that the ³[KP] species can be observed and then it reacts to produce mainly an ArPK radical species by hydrogen abstraction from water molecules in solutions that have less than 50% water. The rate constant of the hydrogen abstraction reaction will be affected by the concentration of water. In solution with water concentration between 50% and 90%, the photoinduced hydrogen abstraction and decarboxylation outcomes of the ³[KP] are two competitive processes that lead to the formation of the corresponding intermediates (the ArPK radical and the triplet protonated biradical carbanion species). For aqueous solutions with more than 90% water, the ³[KP] can be observed at an early time delay (20 ns) and then mainly undergoes decarboxylation to form the triplet protonated biradical carbanion species. Thus, the photo-induced decarboxylation of KP in higher water concentration solution can be (at least in part) a tripletmediated mechanism which leads to formation of the triplet protonated biradical carbanion species. This species has a very long lifetime in 90% water and 90% PBS solutions (with the lifetime reaching to the microsecond time scale in some cases) and is likely an important intermediate that reacts with biological components in biological systems.

Acknowledgements

This work was supported by a grant from the Research Grants Council of Hong Kong (HKU 7035/08P), the award of a Croucher Foundation Senior Research Fellowship (2006–07) from the Croucher Foundation and an Outstanding Researcher Award (2006) from the University of Hong Kong to D. L. P.



Scheme 1 A schematic diagram of the proposed photochemical mechanism of KP in different water concentration solutions is displayed.

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