THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

pubs.acs.org/JPCB

Photochemical Reaction of 2-(3-Benzoylphenyl)propionic Acid (Ketoprofen) with Basic Amino Acids and Dipeptides

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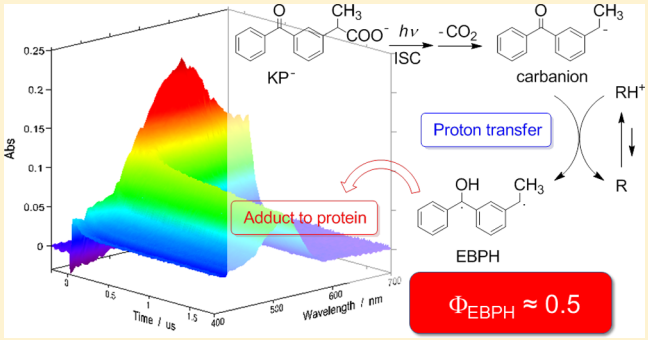
ABSTRACT: Photoreaction of 2-(3-benzoylphenyl)propionic acid (ketoprofen, KP) with basic amino acids (histidine, lysine, and arginine) and dipeptides (carnosine and anserine) including a histidine moiety in phosphate buffer solution (pH 7.4) has been investigated with transient absorption spectroscopy. With UV irradiation KP^- gave rise to a carbanion through a decarboxylation reaction, and the carbanion easily abstracted a proton from the surrounding molecule to yield a 3-ethylbenzophenone ketyl biradical (EBPH). The dipeptides as well as the basic amino acids were found to accelerate the proton transfer reaction whereas alanine and glycine had no effect on the reaction, revealing that these amino acids having a protonated side chain act as a proton donor. The formation quantum yield of EBPH was estimated to be fairly large by means of an actinometrical method with benzophenone, and the bimolecular reaction rate constant for the proton transfer between the carbanion and the protonated basic amino acids or the protonated dipeptides was successfully determined. It has become apparent that the bimolecular reaction rate constant for the proton transfer depended on the acid dissociation constant for the side chain of the amino acids for the first time. This reaction mechanism was interpreted by difference of the heat of reaction for each basic amino acid based on the thermodynamical consideration. These results strongly suggest that the side chain of the basic amino acid residue in protein should play an important role for photochemistry of KP in vivo.



1. INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are known to be a drug providing analgesic, antipyretic, and anti-inflammatory effects. Ketoprofen (2-(3-benzoylphenyl)propionic acid, KP), one of the 2-arylpropionic acids with a benzophenone chromophore (Figure 1), is a very potent NSAID and has been used widely. However, serious side effects such as photocontact dermatitis, which is divided into two categories, phototoxic and photoallergic, have been reported in these decades.[1−10] Photostability, photochemistry, and phototoxicity are key factors in the perspective of pharmacology. The pathogenic mechanism to cause the photocontact dermatitis by NSAIDs with UV irradiation should be unveiled for development of new drugs.

Photochemistry of KP has been extensively studied with experimental and computational methods.[11−19] After the UV irradiation of KP in phosphate buffer solution (pH 7.4), where KP exists as a deprotonated form due to its acidity (pK_a 4.7), KP^- gives rise to a carbanion through a decarboxylation reaction (Figure 2). The carbanion easily abstracts a proton from the surrounding molecule to yield a 3-ethylbenzophenone ketyl biradical (EBPH). The pump−probe experiment with a two-laser system demonstrated that EBPH is produced as the carbanion disappeared.[20] Recently, Phillips' group reported that the proton transfer reaction proceeds in the triplet potential surface by time-resolved resonance Raman spectroscopy coupled with quantum chemical calculations.[19] The series of the photochemical reaction will afford adducts of KP to a protein, and finally cause photocontact dermatitis.

A protein is a polymer of amino acids joined head-to-tail in a long chain. The chain composes a three-dimensional structure that is unique to each type of protein. A NSAID molecule will make a complex with a protein, and in the binding site, where a NSAID molecule exists in a protein a stereoselective photochemistry should take place.[21−27] Monti et al. investigated the interaction of *R*-(−)- and *S*-(+)-KP with bovine serum albumin in buffer solution at neutral pH by circular dichroism, time-resolved fluorescence, and transient absorption spectroscopy.[21−23] They estimated the individual disappearance quantum yields of the 1:1 and 2:1 diastereomeric KP:BSA complexes, and discussed the photoreactivity in the BSA matrix on the basis of diastereoselective photodecarboxylation in the two main protein sites. It is essential to gain information on the reactivity of NSAIDs with amino acids for understanding the

Received: June 13, 2013
Revised: July 17, 2013
Published: July 23, 2013

   dx.doi.org/10.1021/jp405863e | *J. Phys. Chem. B* 2013, 117, 9662−9668

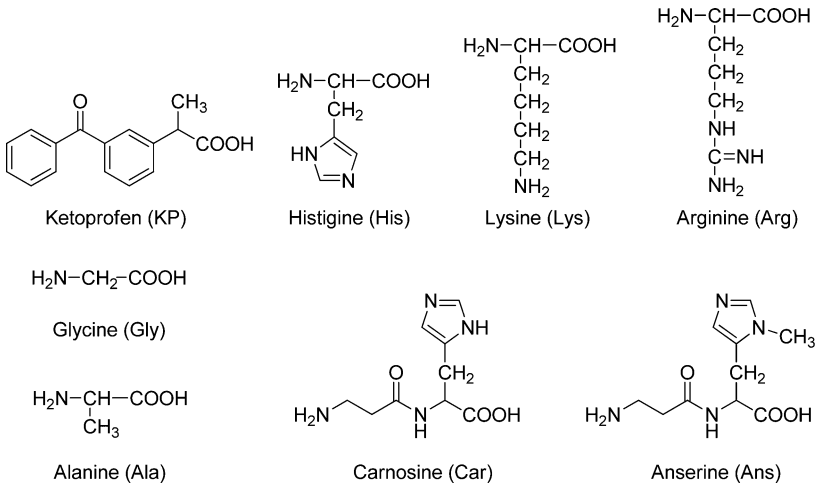
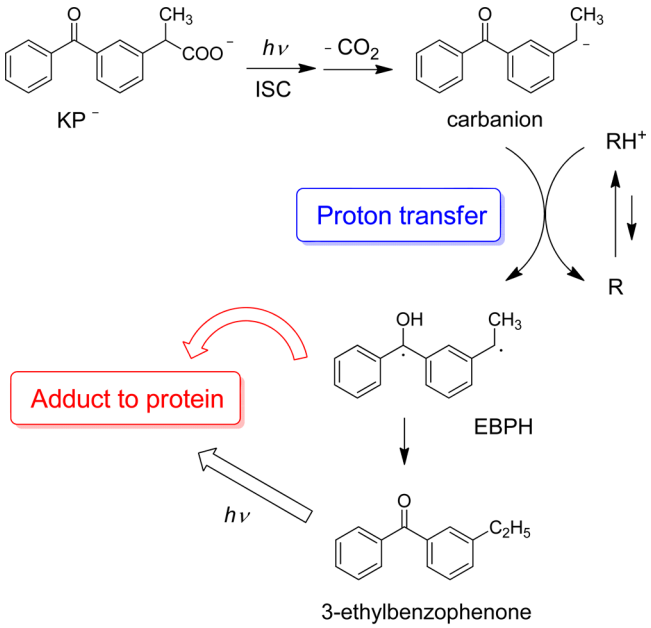


Figure 1. Molecular structures of KP, glycine, alanine, basic amino acids, and dipeptides including a histidine moiety.



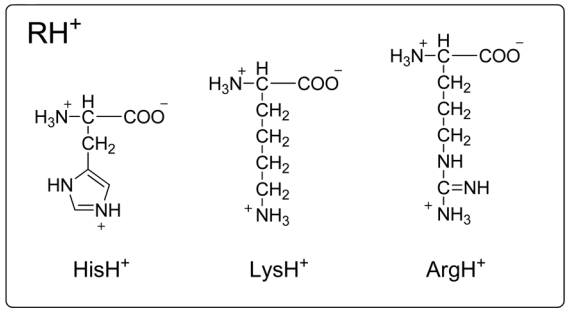


Figure 2. Reaction scheme of excited KP^- with a protonated basic amino acid (RH^+) in phosphate buffer solution (pH 7.4).

pathogenic mechanism causing the photocontact dermatitis by NSAIDs with UV irradiation. In the previous papers, we studied the photochemistry of KP with three basic amino acids, histidine (His), lysine (Lys), and arginine (Arg), in the phosphate buffer solution (pH 7.4).[28,29] The basic amino acids having the protonated side chain, $HisH^+$, $ArgH^+$, and $LysH^+$, were found to accelerate the proton transfer reaction to form EBPH, revealing that the protonated form of these amino acids acts as a good proton donor. Especially, the relative formation quantum yield of EBPH by $HisH^+$ was ca. 40 times larger than that by $ArgH^+$ or $LysH^+$. However, the reactivity and the reaction mechanism of KP with amino acids have not been understood sufficiently.

In this article, we estimated the formation quantum yield of EBPH from KP carbanion by means of an actinometrical method with benzophenone, and the bimolecular reaction rate constant for the proton transfer reaction between the carbanion and the basic amino acids or the dipeptides was successfully determined. The reaction mechanism of KP with basic amino acids will be discussed in detail.

2. EXPERIMENTAL SECTION

KP (Sigma-Aldrich; purity 99.9%), L-histidine (Wako Chemical; GR grade), L-lysine, L-arginine, L-anserine, L-carnosine, L-alanine, and glycine (Sigma-Aldrich; GR grade) were used as received. Distilled water (Kanto Chemical; HPLC grade) was used as a solvent from freshly opened bottles. All phosphate buffer solutions were carefully prepared as the pH values kept to 7.4 (the concentration was 1/15 mol L^{-1}) and were deaerated by argon gas (purity 99.9%) for half an hour before use.

An experimental setup for the transient absorption spectrum measurement has been described elsewhere.[30−32] Briefly, a XeCl excimer laser (Lambda Physik; COMPex102, 308 nm) was used as an excitation light source, and a Xe short arc lamp (Ushio; UXL-300DO, 300 W) was used as a monitoring light. The monitoring light passing through a sample cell (Tosoh Quartz; T514M-ES-10, 10 mm optical path length) was detected with a monochromator (Nikon; P250)/photomultiplier tube (Hamamatsu Photonics; R928) system. The signal was measured by a digital oscilloscope (Sony Tektronix; TDS380P) and transferred to a personal computer. The signal was averaged over 50 laser shots to improve the S/N ratio. The sample solution constantly flowed in the cell to remove the effect of photoproducts. All measurements were carried out at room temperature.

Absorption spectra were measured with a double beam spectrophotometer (Jasco; Ubest V-550).

dx.doi.org/10.1021/jp405863e | J. Phys. Chem. B 2013, 117, 9662−9668

3. RESULTS AND DISCUSSION

3.1. Disappearance of KP Carbanion and Formation of EBPH by Amino Acids and Dipeptides. Figure 3a shows a two-dimensional description of transient absorption of KP (0.70 mM) in phosphate buffer solution (pH 7.4) in the presence of 20 mM carnosine (Car). The spectrum between 450 and 700 nm immediately after the laser irradiation (Figure 3b) was assigned to KP carbanion, produced from $^3KP^{-*}$ through decarboxylation. The quantum yield of the decarboxylation was reported to be as large as 0.75.[11] The spectrum at 1.7 μs has a peak at around 520 nm, which was safely assigned to 3-ethylbenzophenone ketyl biradical (EBPH). The absorption and emission spectra of EBPH were similar to those of a KP ketyl radical (just like a benzophenone ketyl type radical) but slightly blue-shifted.[20] The pump−probe experiment with a two-laser system probed that EBPH was formed as the carbanion disappeared because the rise time of the emission of EBPH corresponded to the lifetime of the carbanion.

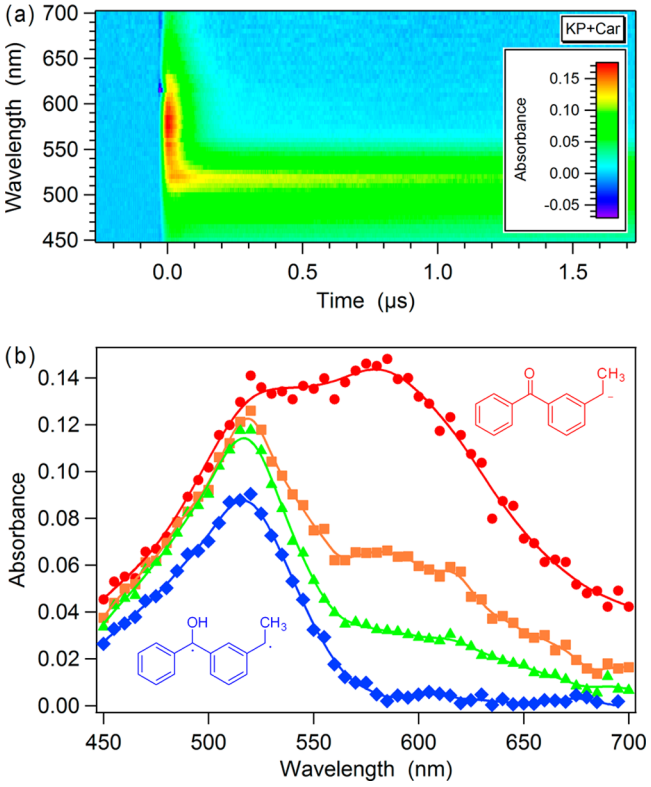


Figure 3. (a) Two-dimensional description of transient absorption of KP (0.70 mM) with Car (20 mM) in phosphate buffer solution (pH 7.4) and (b) the spectra obtained at 0 ns (●, red), 40 ns (■, orange), 100 ns (▲, green), and 1.7 μs (◆, blue) after the laser.

Figure 4 shows time profiles of the transient absorption monitored at 600 nm without (red) and with 20 mM of Car (blue) in phosphate buffer solution (pH 7.4). In the presence of Car, the lifetime of the carbanion obviously became shorter, indicating that some reaction took place between the carbanion and Car. The plots of the decay rate constant of the carbanion ($k_{carbanion}$) against the concentration of Car added are shown in the inset of Figure 4. The apparent bimolecular quenching rate constant, k_q^{app}, was successfully determined to be 4.8×10^8 M^{-1} s^{-1} from the slope of the straight line obtained by the least-squares fitting.

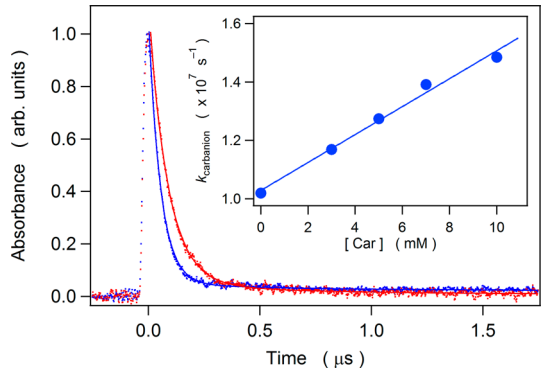


Figure 4. Time profiles of the transient absorption monitored at 600 nm of KP in phosphate buffer solution (pH 7.4) without (red) and with 20 mM of Car (blue). Inset: Plots of the decay rate constant of carbanion ($k_{carbanion}$) against concentration of Car added.

Figure 5 shows time profiles of the transient absorption monitored at 525 nm of KP without (red) and with 20 mM of Car (blue). Two decaying components were observed: The fast one is due to the carbanion and the slow one is due to EBPH. By adding Car, the yield of EBPH obviously increased and the decay of the carbanion was accelerated, revealing that Car should enhance the proton transfer reaction of the carbanion to afford EBPH.

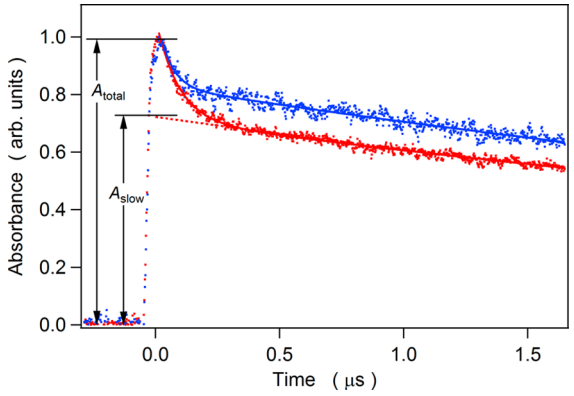


Figure 5. Time profiles of the transient absorption monitored at 525 nm of KP in phosphate buffer solution (pH 7.4) without (red) and with 20 mM of Car (blue). A_{total} and A_{slow} are the total intensity immediately after the laser irradiation and the pre-exponential factor of the slow component, respectively.

Car, which is a dipeptide composed of β-alanine and His, was found to be a proton donor for KP carbanion. The candidates for the proton donating site of Car in the phosphate buffer solution (pH 7.4) are (i) the protonated N terminal (—NH_3^+), (ii) the imidazole —NH— of the side chain, and (iii) the protonated imidazole =NH^+—, which is a minor species in the acid−base equilibrium due to the acid dissociation constant of the His moiety (pK_a = 6.76 for Car[33]) (see Figures 1 and 2). Although the same experiments with alanine and glycine instead of Car were carried out, no effect on both the lifetime of the carbanion and the quantity of EBPH was observed. In the previous work on His, the protonated side chain =NH^+—, not the imidazole —NH—, was shown to be a proton-donating site.[28] Hence, it was concluded that the protonated imidazole side chain of Car should be a proton-donating site for the KP carbanion.

dx.doi.org/10.1021/jp405863e | J. Phys. Chem. B 2013, 117, 9662−9668

Since the minor species of Car having the protonated His side chain, $CarH^+$, is a proton donor for the KP carbanion, the apparent quenching rate constant of the carbanion should be corrected with its concentration. The ratio of $[CarH^+]$ to [Car] was estimated to be 1/4.37 in phosphate buffer solution of pH 7.4 based on the acid dissociation constant of the side chain for Car (pK_a = 6.76) with the following equation:

$$\log\frac{[\mathrm{Car}]}{[\mathrm{CarH}^+]} = \mathrm{pH} - \mathrm{p}K_a \quad (1)$$

The net bimolecular quenching rate constant, k_q^{net}, was successfully estimated to be 2.6×10^9 M^{-1} s^{-1} (= (1 + 4.37) k_q^{app}). The results are listed in Table 1. In a similar fashion, the net quenching rate constant by anserine (Ans), which is a dipeptide composed of β-alanine and *N*-methyl-His, was also obtained. The results with basic amino acids and dipeptides are summarized in Table 1. The k_q^{net} values varied widely from the diffusion-controlled rate constant of water (6.5×10^9 M^{-1} s^{-1} at 293 K[34]) to 1×10^8 M^{-1} s^{-1}, but it was found that the rate constant strongly depends on the pK_a value of the side chain in the amino acids and the dipeptides. In the following section, the formation quantum yield of EBPH and the proton transfer rate constant for the formation of EBPH are estimated.

Table 1. Apparent and Net Quenching Rate Constants of KP Carbanion by Amino Acids or Dipeptides and Formation Rate Constants of EBPH

amino acids or dipeptides	pK_a[a]	k_q^{app} /M^{-1} s^{-1}	k_q^{net} /M^{-1} s^{-1}	k_r[b] /M^{-1} s^{-1}
His	6.04	2.9×10^8[c]	6.7×10^9[c]	4.6×10^9
Car	6.76	4.8×10^8[b]	2.6×10^9[b]	1.9×10^9
Ans	7.04	9.4×10^8[b]	3.1×10^9[b]	1.8×10^9
Lys	10.67	1.1×10^8[d]	1.1×10^8[d]	1.2×10^8
Arg	12.10	1.1×10^8[d]	1.1×10^8[d]	1.3×10^8

[a] $pK_a = -\log K_a$. K_a denotes acid dissociation constant of the side chain of amino acids or dipeptides for aqueous solution at 298 K. Reference 33. [b] This work. [c] Reference 28. [d] Reference 29.

3.2. Determination of Quantum Yield and Proton Transfer Rate Constant for EBPH Formation. As shown in the previous section, increment of EBPH, produced from KP carbanion through proton transfer, was clearly shown by addition of basic amino acids and dipeptides (Figure 5). To obtain the proton transfer rate constant between the carbanion and amino acids or dipeptides, the quantum yield of EBPH was estimated. Since the formation rate constant of EBPH, which equals the disappearance rate constant of carbanion, was two orders larger than the disappearance rate constant of EBPH, the EBPH formation quantum yield can be expressed as

$$\Phi_{EBPH} = \frac{[\mathrm{EBPH}]_0}{[\mathrm{carbanion}]_0} = \frac{A_{slow}}{A_{total}} \cdot \frac{\varepsilon_{carbanion}}{\varepsilon_{EBPH}} \quad (2)$$

where $[carbanion]_0$ and $[EBPH]_0$ are yields of the carbanion and EBPH with an incident laser power, A_{total} and A_{slow} are total and slow-component absorption intensities at t = 0 monitored at 525 nm, and $\varepsilon_{carbanion}$ and ε_{EBPH} are molar absorption coefficients of the carbanion and EBPH at 525 nm, respectively. We measured the laser power dependence of absorption intensity at 525 nm to estimate the molar absorption coefficient of carbanion by the relative actinometrical method with benzophenone (BP) in acetonitrile as a standard. Figure 6 shows the plots of absorbance for BP in acetonitrile and KP in phosphate buffer solution (pH 7.4) measured immediately after the laser irradiation against incident laser power. A good linear relation for both plots was observed. The $\varepsilon_{carbanion}$ value was given by

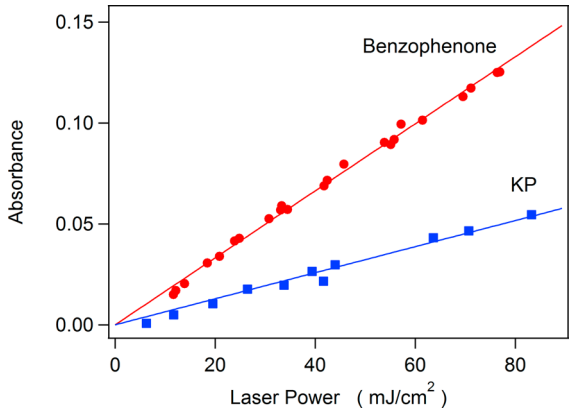


Figure 6. Plots of absorbance for BP in acetonitrile and KP in phosphate buffer solution (pH 7.4) observed immediately after the laser against incident laser power.

$$\varepsilon_{carbanion} = \frac{A_{KP}/I_L}{A_{BP}/I_L} \cdot \frac{\Phi_T^{BP}}{\Phi_{carbanion}} \cdot \varepsilon_{^3BP^*} \quad (3)$$

where A_{KP} and A_{BP} are absorbance monitored at 525 nm immediately after the laser irradiation for KP and BP, respectively, I_L is intensity of the incident laser shot, Φ_T^{BP} is intersystem crossing yield of BP (1.00[34]), $\Phi_{carbanion}$ is formation quantum yield of the carbanion through decarboxylation (0.75[11]), and $\varepsilon_{^3BP^*}$ is molar absorption coefficient at 525 nm of $^3BP^*$ (6250 M^{-1} cm^{-1}, ref 34). From the ratio of the slopes in Figure 6 $[(A_{KP}/I_L)/(A_{BP}/I_L)=0.37 \pm 0.01]$, the $\varepsilon_{carbanion}$ value was determined to be (3100 ± 100) M^{-1} cm^{-1}. By assuming that the ε_{EBPH} value equals that of the benzophenone ketyl radical (BPK) at 545 nm (4600 M^{-1} cm^{-1}, ref 35) because the spectral features of BPK and EBPH are quite similar, the value of Φ_{EBPH} can be obtained. The Φ_{EBPH} value in the absence of Car was successfully estimated to be 0.50. Figure 7 shows the formation quantum yield of EBPH against $CarH^+$ concentration. It became clear that $CarH^+$ leads increment of EBPH.

The proposed reaction scheme for KP carbanion in the presence of the basic amino acid is shown in Figure 8. The formation quantum yield of EBPH from the carbanion can be estimated from the following equation based on the reaction scheme.

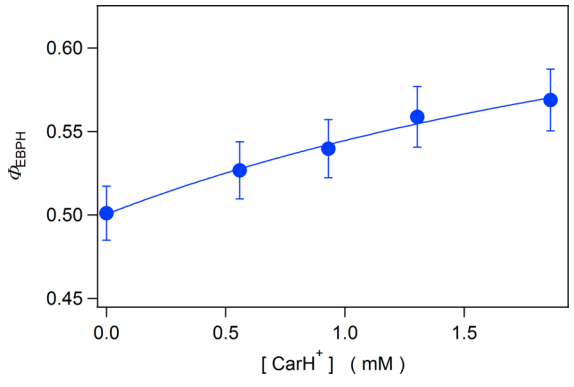


Figure 7. Plots of the Φ_r value against $CarH^+$ concentration.

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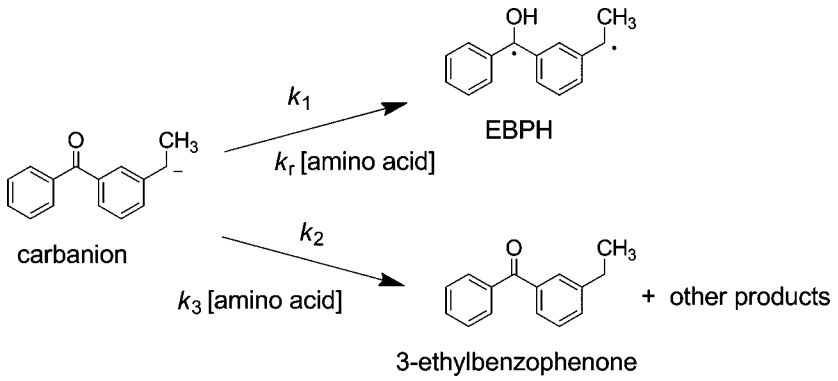

Figure 8. Proposed reaction scheme of KP carbanion.

$$\Phi_{EBPH} = \frac{k_1 + k_r[\text{amino acid}]}{k_1 + k_2 + k_q[\text{amino acid}]} \quad (4)$$

$$k_q = k_r + k_3$$

Here, k_1 and k_r denote the rate constants to form EBPH without and with amino acid, respectively, and k_2 and k_3 are the rate constants for another reaction (for example 2-ethylbenzophenone production) without and with amino acid, respectively. In the case of Car, the concentration of amino acid is $[CarH^+]$.

The experimental results of the Φ_{EBPH} value against the $CarH^+$ concentration shown in Figure 7 were analyzed by eq 4 with $(k_1 + k_2) = 1.0 \times 10^7\ s^{-1}$, $k_q = 2.6 \times 10^9\ M^{-1}\ s^{-1}$, k_1, and k_r used as fitting parameters. The value of k_r by $CarH^+$ was successfully obtained to be $1.9 \times 10^9\ M^{-1}\ s^{-1}$. This large value of k_r in comparison with $(k_1 + k_2)$ and k_q indicates that the proton transfer from the protonated histidine moiety in Car to KP carbanion to afford EBPH is a quite efficient reaction, and that the protonated histidine moiety is a good proton donor. In addition, the reaction to produce EBPH not 3-ethylbenzophenone is the major process, suggesting that the reaction should proceed on the triplet energy surface. This agrees well with the experimental results obtained by time-resolved Raman spectroscopy, which suggested the carbanion would have a triplet multiplicity.[19]

The results obtained by Car as well as other basic amino acids and Ans are summarized in Table 1. Figure 9 shows the logarithmic plots of the k_r value against pK_a. It was found that the k_r value largely depended on the pK_a.

3.3. Reaction Dynamics of Excited KP with Basic Amino Acids and Dipeptides. In the previous section, it was shown for the first time that the bimolecular reaction rate constant for the proton transfer strongly depended on the acid dissociation constant for the side chain of the amino acids. The reaction mechanism will be considered by difference of the heat of reaction for each basic amino acid based on the thermodynamical consideration.

The Brønsted catalysis equation is known to give information about a reaction mechanism. Many general acid catalyzed reactions in the ground state are found to obey the Brønsted catalysis law,[36,37]

$$k \propto K_a^{\alpha} \quad (5)$$

$$\log k = \alpha \log K_a + \text{constant} \quad (6)$$

where k is the catalytic reaction rate constant and K_a is the corresponding acid dissociation constant. The plots of the logarithm of k against the logarithm of K_a for a series of acids give a straight line with slope α. Horiuchi and Polanyi led the following result from the reaction potential curve with the activation energy E_a and the reaction enthalpy ΔH (see Figure 10),

$$\Delta E_a = \alpha \Delta(\Delta H) \quad (7)$$

where ΔE_a and $\Delta(\Delta H)$ are differences of the activation energy and the reaction enthalpy between reaction curves P1 and P2, respectively, and α is the proportionality factor $(0 < \alpha < 1)$. The ΔE_a value equals the product of α and $\Delta(\Delta H)$. The factor α obviously depends on the relative slopes of curves R and P at their points of intersection. The proportionality constant α denotes the extent of reaction at the transition state. Reactions that have low values for α are considered to have a transition state closely resembling the reactant with little proton transfer, while reactions for nearly unity α are to have a transition state closely resembling the product.

The plots of log k_r against pK_a for the side chain of the amino acids and dipeptides (Figure 9) clearly show a linear correlation. This result reveals that the proton transfer reaction between carbanion and protonated amino acids or dipeptides obeys the Brønsted correlation. From the slope of this straight line obtained with the least-squares analysis, the α value was determined to be 0.25 ± 0.04. This indicates that the proton would be located near the side chain of the amino acid in the

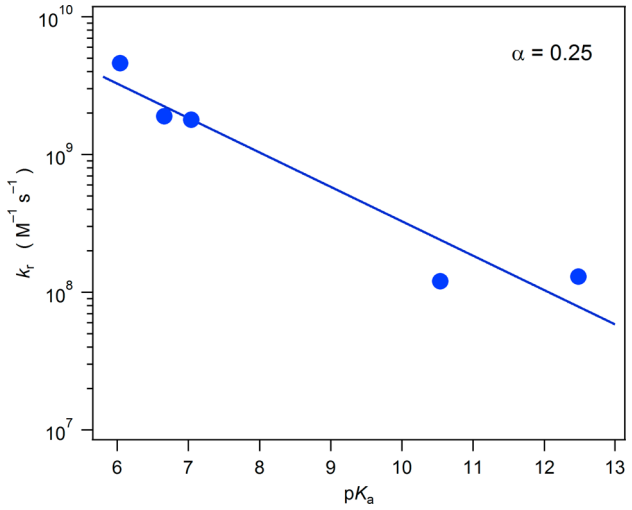

Figure 9. Plots of the k_r value against the pK_a value of the side chain of the amino acids or the dipeptides. The estimated α value was determined to be 0.25 ± 0.04.

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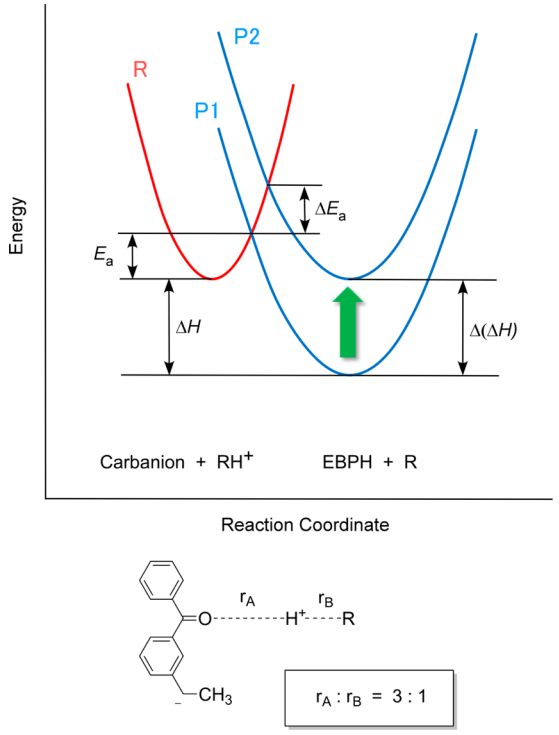

Figure 10. Potential energy curves for the proton transfer reaction between KP carbanion and the protonated amino acids (RH^+) to produce EBPH and R.

transition state. It was concluded that the reaction of the KP carbanion with basic amino acids and dipeptides strongly depends on the acidity of the side chain of the amino acids, resulting from energy and the structure of the transition state. Furthermore, the reaction to produce EBPH not 3-ethylbenzophenone is the major process, suggesting that the reaction should proceed on the triplet energy surface. To obtain further information on the reaction dynamics, quantum chemical calculation on the reaction potential is in progress.

4. CONCLUSION

Photoreaction of KP^- with basic amino acids and dipeptides (carnosine and anserine) including a histidine moiety in a phosphate buffer solution (pH 7.4) has been investigated. The dipeptides as well as the basic amino acids were found to accelerate the proton transfer reaction. The amino acids having a protonated side chain act as a proton donor. The formation quantum yield of EBPH was estimated by means of an actinometrical method with benzophenone, and the bimolecular reaction rate constant for the proton transfer was successfully determined. It was found that the reaction of KP carbanion with basic amino acids and dipeptides strongly depends on the acidity of the side chain of the amino acids, resulting from energy and the structure of the transition state. Since the proportionality factor α was determined to be 0.25 ± 0.04, the proton would be located near the side chain of the amino acid in the transition state. Furthermore, the reaction to produce EBPH not 3-ethylbenzophenone is the major process, suggesting that the reaction should proceed on the triplet energy surface. To obtain further information on the reaction dynamics, quantum chemical calculation on the reaction potential is in progress. These results strongly suggest that the side chain of the basic amino acid residue in protein should play an important role for the photochemistry of KP in vivo.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The present work was financially supported in part by a Grant-in-Aid for Scientific Research (C22550007) (KAKENHI) from Japan Society for the Promotion of Science (JSPS).

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