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Excited State Chemistry of Aromatic Amino Acids and Related Peptides. II. Phenylalanine

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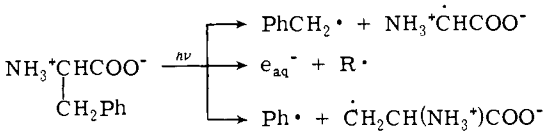
Abstract: A pulsed frequency quadrupled neodymium laser emitting at 265 nm (~15 nsec duration) was used to optically excite phenylalanine and derivatives in water at 25°. The following systems were studied: benzene, toluene, phenylpropionic acid, phenylalanine, *N*-acetylphenylalanine amide, phenylalanine amide, and glycylphenylalanylglycine. The triplet-triplet absorption spectra of most of these compounds were observed and their lifetimes determined. These lifetimes are ~1-3 μsec and are shorter lived than those observed for the corresponding tyrosine derivatives. The triplet states are quenched by oxygen with $k_q \sim 3\text{-}5 \times 10^9\ M^{-1}\ \text{sec}^{-1}$. The photoionization and photodissociation of phenylalanine were studied as a function of pH and temperature. The photoionization of phenylalanine and derivatives has the triplet state as the main precursor, and electron ejection occurs in all cases via a biphotonic process. The $\phi_{e_{aq}^-}$ is dependent on the state of protonation of the NH_3^+ group, and decreases when the α-amino group is present as NH_2. The photodissociation reactions occur, at all pH values, primarily via the triplet state as precursor. The dissociation processes can be biphotonic or monophotonic, depending on certain conditions which have been defined. In proteins, phenylalanine is expected to dissociate via a monophotonic process. The photoionization of benzene in water is reported. These and other results are discussed.

On the basis of the singlet excited state and the triplet state energies of phenylalanine, it has generally been considered that the sequence of electronic energy transfer from phenylalanine → tyrosine → tryptophan was feasible and probable in protein macromolecules containing these aromatic amino acids.[1,2] However, the destruction of phenylalanine (Phe) occurs on uv irradiation of proteins.[3] Based on the low extinction coefficient of Phe and its blue-shifted absorption spectrum compared to tyrosine (Tyr) and tryptophan (Trp), there is, however, a low probability for direct optical excitation of Phe in proteins which contain a number of Tyr and Trp molecules.

The fluorescence lifetime and quantum yield of Phe ($\sim 10^{-3}\ M$, 20°) in aqueous solution[4-6] are $\phi_F = 0.025$ and $\tau_F = 6.8$ nsec. A marked temperature dependence was found,[5] with ϕ_F and τ_F decreasing with increasing temperature in the range 2-68°. This deactivation process was indicated[3] to be due mainly to internal conversion and only to a small extent due to intersystem crossing. The ϕ_F decreases[4] by ~30% on ionization of the COOH group and ~15% on ionization of the NH_3^+ group. The phosphorescence of Phe has been observed[1] only in glasses at 77°K and has a band maximum at 385 nm and $\tau_P \sim 5.5$ sec.

Flash photolysis studies[7] of Phe in water at 20° showed that: (a) the photodissociation process leading to the formation of the benzyl radical and the photoionization process leading to the hydrated electron e_{aq}^- were both strongly dependent on the state of ionization of the free end groups COOH and NH_3^+, and in particular to the amino group (these processes followed the pK_a of the ground state molecule); (b) the excited state precursors of both processes were long lived, and probably the triplet states; (c) the photoionization process was biphotonic in nature; (d) in neutral and acid solutions, the photodissociation processes from Phe, Phe-NH_2, and *N*-Ac-Phe were biphotonic, whereas in alkaline solutions (above the pK_a of NH_3^+) only one quantum was required to bring about the same photodissociation reaction; (e) the triplet state was also involved in alkaline solutions.



$$NH_3^+CH(CH_2Ph)COO^- \xrightarrow{h\nu} PhCH_2\cdot + NH_3^+\dot{C}HCOO^- \quad (1)$$

$$\xrightarrow{h\nu} e_{aq}^- + R\cdot \quad (2)$$

$$\xrightarrow{h\nu} Ph\cdot + \dot{C}H_2CH(NH_3^+)COO^- \quad (3)$$

The triplet state of Phe and related compounds was not observed,[7] presumably due to its relatively short lifetime. Reported below is a laser photolysis study of Phe and phenylalanine peptides in water using a quadrupled neodymium laser emitting at 265 nm with single pulses of ~15 nsec duration. The triplet-triplet absorption spectra of β-phenylpropionic acid, toluene, Phe, *N*-Ac-Phe, *N*-Ac-Phe-NH_2, Phe-NH_2, and Gly-Phe-Gly were observed and their lifetimes were determined. The pH and temperature dependences of the photoionization and photodissociation processes were examined, and the mechanisms leading to these dissociations are discussed in detail. Preliminary results have been presented.[8]

Experimental Section

Details of the experimental set-up and conditions used have been given elsewhere.[9] Briefly, a quadrupled neodymium laser was used, emitting at 265 nm, with single pulses of ~15 nsec duration. The monitoring light source was a 250 W Xenon lamp whose current was boosted for ~1 msec, resulting in an increase in light output at $\lambda < 280$ nm by a factor of >400. On-line analysis of the data was carried out using a Biomation 8100 and a Hewlett Packard 9830A calculator.

The chemicals used were the best research grade available commercially and were obtained from Cyclochemicals, Calbiochem, Sigma Chemicals, and Fox Chemicals. They were used as received. Standard reagents and chemicals were obtained from Baker and Adamson, Eastman, Mallinckrodt, and Aldrich.

Solutions were buffered using perchloric acid, potassium hydroxide, ~0.2 mM phosphates, and 1.0 mM borate buffers. Fresh samples were used for each laser pulse.

Actinometry was based on anthracene in cyclohexane solutions. The T-T absorption of anthracene was monitored at 428 nm (using narrow slits on the monochromator), $\epsilon_{428} = 6.47 \times 10^4\ M^{-1}\ \mathrm{cm}^{-1}$ (from ref. 10) and a $\phi_{\mathrm{ISC}} = 0.75$ (from ref 11).

Results and Discussion

Phenylalanine and Related Compounds. Using 265 nm light, only the ${}^1A_{1g} \rightarrow {}^1B_{2u}$ benzene transition of phenylalanine and related compounds and peptides is optically excited. The vibronic structure of this absorption band in water has a maximum at ~258 nm and $\epsilon_{258} \sim 190\ M^{-1}\ \mathrm{cm}^{-1}$. Changes in the spectrum of phenylalanine with pH have been observed[12] indicative[13,14] of strong intramolecular interactions and are suggested to be charge transfer in character (see more below).

On laser photolysis of oxygen-free aqueous solutions of phenylalanine ($\mathrm{p}K_a{}^1 = 1.83$ and $\mathrm{p}K_a{}^2 = 9.13$) at pH 7.5, a transient optical absorption spectrum is observed immediately after the 15 nsec laser pulse (Figure 1b). Absorption maxima at ~318, ~307, and ~245 nm are observed at ~20 nsec after the pulse. A weak absorption with $\lambda_{\max} \sim 720$ nm, assigned to the hydrated electron e_{aq}^-, was found but is not shown in Figure 1b. At 10 μsec after the pulse a different transient spectrum is observed (Figure 1b). The difference in the two spectra is represented by the full line in Figure 1b.

The 10 μsec spectrum resembles that previously observed[7] under flash photolytic conditions (time resolution ~10 μsec) which was assigned primarily to the benzyl and $\mathrm{NH_2\dot{C}HCOO^-}$ radicals (reaction 1). The spectrum of the benzyl radical was recently redetermined,[15] and maxima at 318, 307, and 258 nm were observed, under similar experimental conditions, with extinction coefficients of 9.0×10^3, 4.7×10^3, and $2.5 \times 10^4\ M^{-1}\ \mathrm{cm}^{-1}$, respectively. The glycine radical has a $\lambda_{\max} \sim 250$ nm and an extinction coefficient which is strongly dependent upon the pH and the acid-base properties of this radical.[16]

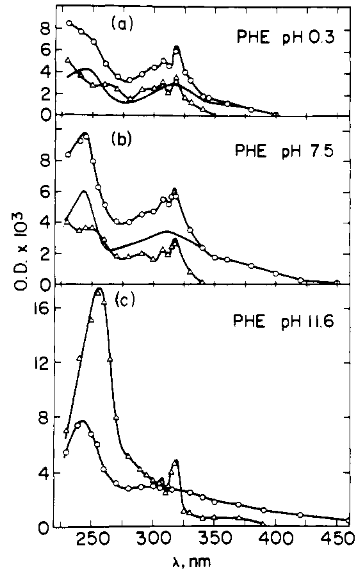


Figure 1. Absorption spectra of the transient species produced on optical excitation at 265 nm of phenylalanine (4.0 mM) in water at 25°: (a) at pH 0.3, (b) at pH 7.5, and (c) at pH 11.6. OD were read at 20 nsec (○) and 10 μsec (△) after the 15 nsec laser pulse. The difference spectrum represented by a full line is the T-T absorption of phenylalanine at the particular pH. Ground state OD at 265 nm was identical in each case. In (b) and (c) solutions contained $\mathrm{N_2O}$ (1 atm) and 1.0 M *tert*-butyl alcohol to scavenge the optically generated hydrated electrons (see text).

The 20 nsec spectrum also shows the presence of the benzyl and glycine radicals, in addition to another transient absorption. This absorption with maxima at ~310 nm and ~243 nm is suggested to be the triplet-triplet absorption spectrum of Phe at pH 7.5 (difference spectrum in Figure 1b). This assignment is based on the following observations: (a) the decay kinetics at ~310 and ~240 nm are first order with $k = 3.2 \pm 0.3 \times 10^5\ \mathrm{sec}^{-1}$ (see Table I); (b) the T-T spectrum of Phe is quenched by oxygen with $k_q = 3.3 \pm 0.3 \times 10^9\ M^{-1}\ \mathrm{sec}^{-1}$ (see Table II); (c) similar T-T spectra and lifetimes have been observed from toluene, β-phenylpropionic acid, and Phe peptides in water (see below and Tables I and II).

On optical excitation of Phe at pH 0.3, similar transient spectra were observed (Figure 1a). The shift in the absorption maxima and relative extinction coefficients probably reflect (a) the change in the ϕ of the photoprocesses 1-3

Table I. Lifetimes of Triplet States of Phenylalanine and Related Compounds in Water at 25°

Substrate[b]	pK_a	pH	λ monitored, nm	k, sec⁻¹	τ
Phenylalanine (3.8 mM)	1.8, 9.1	7.5	240, 320	$3.2 \pm 0.3 \times 10^5$	3.1 μsec
		11.6	260,[a] 360	$5.0 \pm 1.0 \times 10^5$	2.0 μsec
		0.3	240	$4.1 \pm 0.4 \times 10^5$	2.4 μsec
Phenylpropionic acid (5.0 mM)	4.7	7.3	240	$2.0 \pm 0.2 \times 10^5$	5.0 μsec
Toluene (3.0 mM)		7.0	240	$3.6 \pm 0.2 \times 10^5$	2.8 μsec
N-Acetylphenylalanine (3.8 mM)	~3.6	8.1	260[a]	$4.7 \pm 0.4 \times 10^5$	2.1 μsec
		1.4	260[a]	$9.6 \pm 0.6 \times 10^5$	1.0 μsec
N-Acetylphenylalanineamide (3.0 mM)		8.4	255,[a] 360	$5.2 \pm 0.3 \times 10^5$	1.9 μsec
Phenylalanineamide (5.0 mM)	7.2	12.5	260[a]	$8.0 \pm 1.5 \times 10^5$	1.2 μsec
Glycylphenylalanylglycine (2.5 mM)		5.0	260[a]	$5.7 \pm 0.8 \times 10^5$	1.8 μsec
		11.1	260[a]	$6.5 \pm 1.5 \times 10^5$	1.5 μsec
		0	260[a]	$5.2 \pm 1.0 \times 10^5$	1.9 μsec

[a] At this wavelength, the transient absorption increases with the decay of the triplet due to the formation of transient species having higher extinction coefficients (see text). [b] Lifetimes were determined at the substrate concentrations given in parentheses.

Table II. Quenching of Triplet States of Phenylalanine and Related Peptides in Water at 25°

Aromatic amino acid[a]	pH	Quencher	k_q, M^{-1} sec^{-1}
Phenylalanine (3.8×10^{-4} *M*)	7.5	O_2	$3.3 \pm 0.3 \times 10^9$
Phenylalanine (3.4×10^{-4} *M*)	11.6	O_2	$4.5 \pm 1.0 \times 10^9$
Phenylalanine (3.8×10^{-4} *M*)	7.5	Ni^{2+}	$4.1 \pm 0.2 \times 10^7$
Phenylalanine ($0.1-2 \times 10^{-2}$ *M*)	7.5	Phe	$<2.0 \times 10^6$
N-Acetylphenylalanine (3.8 m*M*)	8.1	O_2	$3.9 \pm 0.5 \times 10^9$
Glycylphenylalanylglycine (2.5 m*M*)	5.8	O_2	$3.8 \pm 0.5 \times 10^9$

[a] Quenching rates determined at substrate concentrations given in parentheses.

and (b) the change in the spectra of the T-T absorption and the radicals due to their acid-base properties.

On optical excitation of Phe at pH 11.6, a significantly different spectrum is observed at 20 nsec after the pulse, Figure 1c. The spectrum is not "structured", indicating the absence of $PhCH_2\cdot$ radicals, and has maxima at ~243 and ~310 nm. A strong increase in absorbance is observed 10 μsec later, with the observation of the characteristic spectrum of the $PhCH_2\cdot$ radical. The initial 20 nsec spectrum is assigned to the T-T spectrum of Phe at pH 11.6 (one cannot exclude the possibility that other transient species may also be formed and contribute to this spectrum). It should be noted that the rate of formation of $PhCH_2\cdot$ radical is identical to the rate of decay of the triplet state at 360 nm.

pH Dependence. From Figure 1 it is clear that the absorbances at different wavelengths are markedly dependent upon pH. Based on the assignments given to these absorption bands, titration curves for e_{aq}^-, initial T-T, and the benzyl radical (other radicals also absorb at 250 nm) were determined and are shown in Figure 2. The "initial T-T" represents the absorbance measured directly after the pulse and may include radicals produced within the laser pulse duration.

The initial T-T absorption at 250 nm shows an ~30% decrease in acid solutions and an ~24% decrease in alkaline solutions (there is an implied assumption here that the extinction coefficient of the initial T-T Phe at 250 nm is independent of the state of protonation of the molecule, which is probably not entirely valid). The midpoints of this titration curve give values which are very close to the pK_a values of ground state phenylalanine. The initial T-T titration curve also follows very closely the fluorescence curve[4,13] for Phe. The lifetime of ^{3}Phe was found to be only slightly dependent upon pH with τ = 2.4, 3.1, and 2.0 μsec at pH 0.3, 7.5, and 11.6, respectively (see Table I).

The quantum yield for photoionization of Phe is only slightly dependent upon pH (Figure 2) with the yield decreasing by ~17% in alkaline solutions above $pK_a^2 = 9.13$. Due to reaction 4, $k_4 = 2.3 \times 10^{10}$ M^{-1} sec^{-1} (ref 17), it

$$e_{aq}^- + H^+ \longrightarrow H \qquad (4)$$

was not possible to monitor the yield of e_{aq}^- at 650 nm at pH <3, and establish whether the $\phi_{e_{aq}^-}$ decreases in acid solutions. Since e_{aq}^- is formed via the absorption of a second quantum by ^{3}Phe (see below), it follows that $\phi_{e_{aq}^-}$ will reflect the ϕ_{T-T}.

The titration curve at 250 nm of the transient species present *after* the decay of the triplet (as that of the initial T-T and the fluorescence) also follows closely the ground state pK_a values of Phe. However, in this case, a very strong increase in absorbance is observed in alkaline solutions. The major increase reflects the increased yield of $PhCH_2\cdot$ radicals (i.e., the photodissociation process 1) and, only in small part, the change in the extinction coefficients of the acid-base forms[18] of the additional radicals produced. Such an increase in radical yield with pH was also reported[7] in the flash photolysis of Phe.

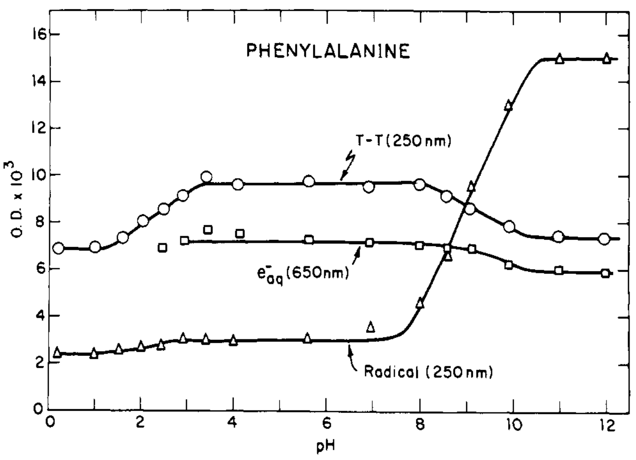


Figure 2. Titration curves of the transient species produced on optical excitation of phenylalanine (4.0 m*M*) at 25°. OD measured at 20 nsec (○, □) and at 10 μsec (△) after the laser pulse.

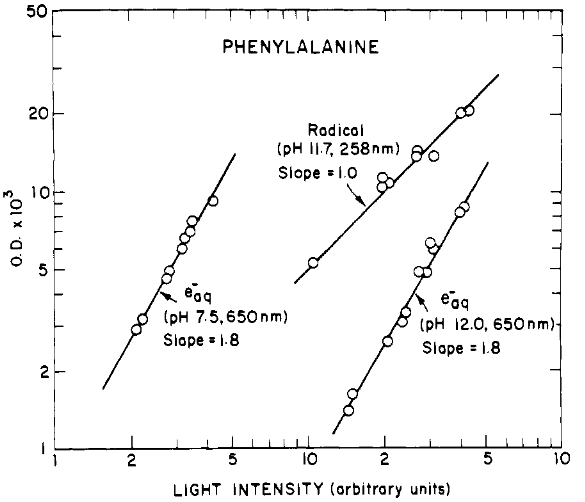


Figure 3. Dependence upon 265 nm light intensity (using a 15 nsec laser pulse) of the yields of transient species produced from phenylalanine (4.0 m*M*, 25°) in water at pH 7.5, 11.7, and 12.0.

Dependence of Yields upon Light Intensity. The flash photolysis study[7] showed that the photodissociation process of Phe was dependent upon I^2 (I = light intensity) in neutral solution, but was $\propto I$ in alkaline solution above pK_a = 9.13. These experiments were repeated using laser excitation of Phe at 265 nm, see Figure 3. The photoionization process, reaction 2, is proportional to I^2 in both neutral and alkaline solutions, see Figure 3. This difference in the dependence upon light intensity supports the conclusion reached earlier[7] that parallel photolytic processes give rise to dissociation and ionization reactions.

Dependence of Yields upon Temperature. Fluorescence studies[5] on Phe in neutral aqueous solutions showed a marked decrease in quantum yield with increasing temperature, from ϕ_F = 0.04 at 2° to ϕ_F = 0.008 at 70°.

Figure 4 shows the dependence upon temperature of the yields of triplets, e_{aq}^-, and radicals formed at pH 7.7 and 11.9. At pH 11.9 the dependence of these yields upon temperature is more marked than at pH 7.7. It should be pointed out that at 650 nm the extinction coefficient of e_{aq}^- decreases with increase in temperature.[17] Hence, $\phi_{e_{aq}^-}$ decreases more sharply with increasing temperature than indicated in Figure 4.

Excited State Chemistry of Phenylalanine. From the laser

Table III. Relative Yields of Photoionization and Photodissociation Processes of Phenylalanine and Related Compounds Optically Excited at 265 nm in Water at 25°[a]

Substrate	pH	e_{aq}^- $\phi_{e_{aq}^-} \times 10^2$	e_{aq}^- Ratio[b]	Ratio OD_{240}[b,c]	Ratio OD_{318}[b,d]
Phenylalanine	7.5	3.4	1.0	1.0	1.0
	11.1	2.8	0.8_2	0.8	1.7
Phenylpropionic acid	7.3	3.4	1.0		
Toluene	7.0	4.1	1.2		
Benzene	7.0	2.4	0.7		
N-Acetylphenylalanine	7.5, 11.2	3.6	1.0_6	1.2	2.6
N-Ac-Phe-NH_2	7.5, 10.5	3.2	0.9_4	0.9	2.0
Phenylalanineamide	5.2	3.2	0.9_4	0.9	0.6
	12.5	2.7	0.8	0,8	1.9
Gly-Phe-Gly	5.0	3.8	1.1	0.9	1.8
	12.5	3.4	1.0	1.3	2.6

[a] Determined from solutions whose absorbance at 265 nm was 0.50 ± 0.05. [b] Ratio based on Phe at pH 7.5, $\phi = 1.0$. [c] OD at ~20 nsec after the laser pulse. [d] OD read after the decay of the triplet at ~10 μsec after the laser pulse.

photolysis results presented above, the following comments and conclusions can be made. (a) On the basis of the transient optical absorption spectra observed and their identification, all the photoprocesses appear to have the triplet state as their main precursor. (b) The lifetime of the triplet state of Phe in water is ~2-3 μsec and its quenching rate constant by ground state Phe is $<2.0 \times 10^6$ M^{-1} sec^{-1}. Toluene and β-phenylpropionic acid in water at pH ~7.0 also have triplet lifetimes of a few microseconds. It is interesting to point out that ^{3}Tyr has[9] a $\tau = 10$ μsec in water at pH 6.0. The quenching rate constant by O_2 is close to diffusion controlled, and similar to that found for ^{3}Tyr (ref 9) and ^{3}Trp (ref 19). Contrary to what was suggested,[7] the quenching of ^{3}Phe by $Ni(ClO_4)$ is relatively slow, $k_q = 4.1 \pm 0.2 \times 10^7$ M^{-1} sec^{-1}. Hence the observed[7] decrease in $PhCH_2\cdot$ radicals by Ni^{2+} is probably due to a fast reaction ($k \geq 10^9$ M^{-1} sec^{-1}) between $PhCH_2\cdot$ and Ni^{2+} ions, and not to the quenching of the triplet state percursor of $PhCH_2\cdot$ radicals. (c) Photodissociation process: in neutral and acidic solutions, photodissociation reactions occur from the triplet state via a biphotonic mechanism. This explains the observed formation of $PhCH_2\cdot$ within the duration of the laser pulse (15 nsec) and the photoflash[7] (~10 μsec) and its dependence upon I^2. In alkaline solutions, no benzyl radicals are formed *during* the laser pulse (Figure 1c) but are produced from the decay of ^{3}Phe. The rate of decay of ^{3}Phe is identical to the rate of formation of $PhCH_2\cdot$. The photodissociation reaction is therefore monophotonic and shows a stronger dependence upon temperature, as expected. The increased yield of radicals at pH 11 is consistent with the more efficient monophotonic process, and does not necessarily reflect an increased ISC in alkaline solutions. Furthermore, a strong temperature dependence (Figure 4b) is consistent with a monophotonic process which is dependent upon activation energy. (d) Photoionization process: the dependence of this process upon the excitation light intensity under both laser and flash photolysis conditions points to the triplet state as the precursor, which absorbs a second quantum of light. The formation of e_{aq}^- occurs within the 15 nsec laser pulse duration. The strong temperature dependence of $\phi_{e_{aq}^-}$ at pH 7.7 and 11.9 appears at first surprising for a biphotonic process. However, since the population of the precursor of the triplet state is equally temperature dependent, $\phi_{e_{aq}^-}$ should follow a similar dependence.

It was suggested[7] that the ejected electron came from the COO^- group in Phe as a result of energy transfer to the carboxyl group

$$^3PhCH_2(NH_3^+)COO^- \longrightarrow PhCH_2(NH_3^+)COO\cdot + e_{aq}^- \quad (5)$$

$$^3PhCH_2(NH_3^+)COO\cdot \longrightarrow PhCH_2NH_3^+ + CO_2 \quad (6)$$

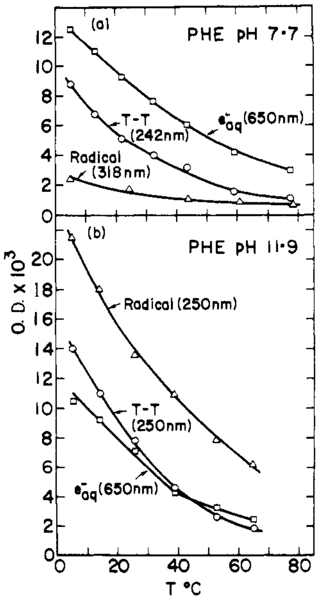


Figure 4. Dependence upon temperature of the yields of the transient species produced from optical excitation of phenylalanine in water at (a) pH 7.7, 4.0 m*M*, and and (b) at pH 11.9, 4.0 m*M*. OD were measured at 20 nsec (○, □) and at 10 μsec (△) after the laser pulse.

No evidence could be found in support of reactions 5 and 6. Also no transient absorption could be observed which could be assigned to an intermediate produced from the ionization of the benzene ring itself. Consequently, no mechanism is presently offered for the site of ejection of electrons from phenylalanine.

Benzene, Toluene, and Phenylpropionic Acid. The ϕ_F of benzene at 25° has been reported to be 0.005[5] and 0.006,[20] and $\phi_T = 0.07$.[21] The triplet-triplet absorption of benzene in water was not observed on laser excitation at 265 nm. The photoionization of benzene (~2×10^{-2} *M*) in water at pH 7.0 was, however, observed and the $\phi_{e_{aq}^-} = 0.024$ (Table III).

Laser photolysis of toluene (3.0 m*M*) in water gave rise to a relatively weak absorption which is assigned to the T-T spectrum of toluene. It decays with $k = 3.6 \pm 0.2 \times 10^5$ sec^{-1} and is effectively quenched by oxygen. Hydrated electrons are also observed and are formed during the laser pulse with $\phi_{e_{aq}^-} = 0.04$. The formation of e_{aq}^-[7,22] and $PhCH_2\cdot$[23] radicals have previously been reported in flash photolysis studies. The ϕ_F for toluene is 0.13 in ethanol[13] and $\phi_T = 0.52$ in isooctane[24] solutions. Due to experimental difficulties, no attempt was made to determine the excited state precursor of the photoionization of toluene. However, based on the conclusions reached for other aromatic[25] and

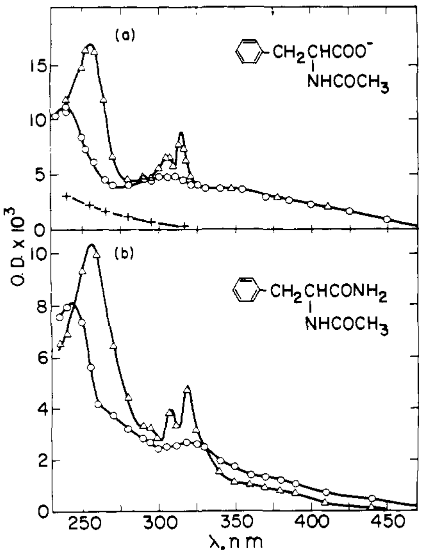
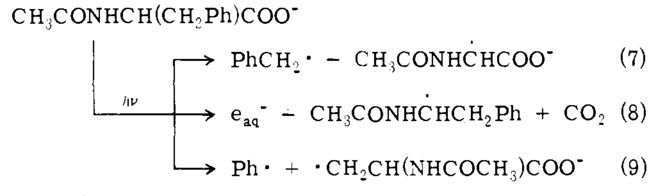


Figure 5. Absorption spectra of the transient species produced on optical excitation at 265 nm in water of (a) 4.0 mM N-acetylphenylalanine at pH 8.1 and (b) 4.0 mM N-acetylphenylalanineamide at pH 8.4. Solutions contained N_2O (1 atm) and 1.0 M t-BuOH (see text). Absorbances were read at 20 nsec (○) and 10 μsec (△) after the pulse. The symbol + is the spectrum obtained in O_2 (1 atm) read at 1.0 μsec after the pulse.

phenolic[26] compounds, it would seem reasonable to assume that photoionization of toluene in water occurs from the triplet state via a biphotonic process.

The flash photolysis of phenylpropionic acid in water was examined.[25] The photodissociation reaction (to produce $PhCH_2\cdot$ and $\cdot CH_2COO^-$ radicals) and the photoionization reaction (to form e_{aq}^-) were shown[25] to occur from the triplet state via a biphotonic mechanism. The triplet state of phenylpropionic acid in water at pH 7.3 was found to decay with $k = 2.0 \pm 0.2 \times 10^5$ sec^{-1} (Table I) and $\phi_{e_{aq}^-} = 0.034$ (see Table III). The ϕ_F of phenylpropionic acid in ethanol was found[13] to be 0.13.

Phenylalanine Peptides. N-Acetylphenylalanine. The state of protonation of the α-amino group in Phe was found to have a considerable effect on the photochemical decomposition, quantum yield, and its mechanism. The flash photolysis[7] of N-acetylphenylalanine (Ac-Phe, pK_a ~ 3.6) at pH 8.5 was explained on the basis of reactions 7 and 8, with reaction 9 assumed to occur.



$$CH_3CONHCH(CH_2Ph)COO^-$$

$$\xrightarrow{h\nu} PhCH_2\cdot + CH_3CONH\dot{C}HCOO^- \quad (7)$$

$$\xrightarrow{h\nu} e_{aq}^- + CH_3CONH\dot{C}HCH_2Ph + CO_2 \quad (8)$$

$$\xrightarrow{h\nu} Ph\cdot + \cdot CH_2CH(NHCOCH_3)COO^- \quad (9)$$

The laser photolysis of 4 mM Ac-Phe in water at pH 8.1 in the absence of oxygen produces immediately after the 15 nsec pulse a transient spectrum with maxima at ~355, ~305, and 240 nm, but with no indication of the presence of the characteristic absorption spectrum of the $PhCH_2\cdot$ radical. After the pulse, an increase in absorbance is observed and is fully developed at ~10 μsec later, see Figure 5a. The $PhCH_2\cdot$ spectrum can now be observed. It is believed to be produced from the decay of the triplet state of Ac-Phe although no actual decay corresponding to ^{3}Ac-Phe could be observed, presumably because the radicals formed from it have stronger absorptions. The absorption of the $PhCH_2\cdot$ radical (at 260 nm) was formed with a $k = 4.7 \pm 0.4 \times 10^5$ sec^{-1} at pH 8.1 and with a $k = 9.6 \pm 0.6 \times 10^5$ sec^{-1} at pH 1.4 in 4 mM solutions. In O_2-saturated solutions a net decay at 240 nm, due to the quenching of ^{3}Ac-Phe, was observed and $k_q = 3.9 \pm 0.5 \times 10^9$ M^{-1} sec^{-1} was derived at pH 8.1.

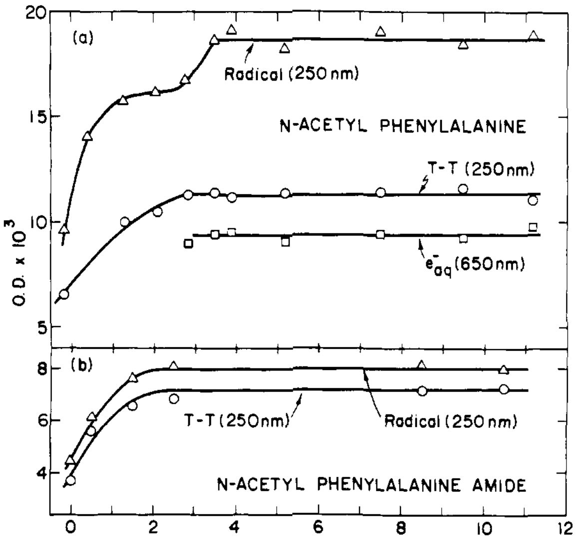


Figure 6. Titration curves of the transient species produced on optical excitation of (a) N-acetylphenylalanine (4.0 mM) and (b) N-acetylphenylalanineamide (4.0 mM). OD were read at 20 nsec (○, □) and at 10 μsec (△) after the pulse.

In addition to the spectra shown in Figure 5a, the e_{aq}^- spectrum with λ_{max} ~ 720 nm was also observed.

The photoionization process, reaction 8, occurs from the triplet state via a biphotonic mechanism.[7] The e_{aq}^- observed was formed within the 15 nsec laser pulse. The 355 nm band observed immediately after the laser pulse, and present in the 10 μsec spectrum, is probably associated with the formation of e_{aq}^-. It was suggested[7] to be the $CH_3CONH\dot{C}HCH_2Ph$ radical.

The photodissociation process, reaction 7, to form $PhCH_2\cdot$ clearly occurs from the decay of the triplet state and is therefore monophotonic in nature.[27]

On photolysis of Ac-Phe in oxygen-saturated solution, a weak spectrum is observed ~1.0 μsec after the laser pulse, Figure 5a. The radicals produced in reactions 7-9 react with O_2 to form peroxy radicals which have absorption maxima below ~250 nm and relatively low extinction coefficients.[28]

Figure 6a shows the pH dependence of the yields of e_{aq}^-, triplet, and radicals produced from the laser photolysis of 4 mM Ac-Phe in water. It is interesting to note the following points: (a) between pH 3.5 and 11.2 the yields of e_{aq}^-, radicals, and triplet are independent of pH (compare with Figure 2 for Phe); (b) the yields of e_{aq}^- and of radicals are at least as high as those observed from Phe at any pH (see Table III); (c) the decrease in absorbance of the radical with decrease in pH is due to protonation of the COO^- group. A similar decrease is observed for N-acetylphenylalanineamide; see Figure 6b.

N-Acetylphenylalanineamide. This molecule has no acid-base properties and is a model compound for Phe in proteins. Laser photolysis of 4.0 mM Ac-Phe-NH_2 at pH 8.4 gives rise to transient spectra similar to those observed for Ac-Phe; see Figure 5b.

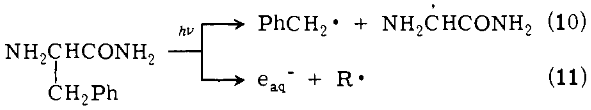
Photoionization of Ac-Phe-NH_2 gives on laser photolysis a $\phi_{e_{aq}^-} = 0.032$. The e_{aq}^- is produced during the pulse and is presumably formed from the triplet state via a biphotonic mechanism. The absence of a COO^- group in this molecule would seem to argue against the earlier suggestion[7] that the ejected electron came from the ionized carboxyl group.

Electron ejection from the aromatic ring followed by an intramolecular charge transfer rearrangement is now tentatively suggested.

The photodissociation process leading to the formation of $PhCH_2\cdot$ radicals occurs from the decay of the triplet state via a monophotonic mechanism. From the rate of formation of $PhCH_2\cdot$, which matches the decay at ~360 nm, one can obtain the decay rate of ^{3}Ac-Phe-NH_2, $k = 5.2 \pm 0.3 \times 10^5$ sec^{-1} (Table I).

Figure 6b shows the pH dependence of the yields of radicals and triplet. These are interpreted in a way similar to that suggested above for Ac-Phe.

Phenylalanineamide. The transient spectrum observed in the flash photolysis of Phe-NH_2 ($pK_a = 7.22$) at pH 9.2 was accounted[7] for on the basis of reaction 10. Laser photolysis at pH 12.5 shows the formation of e_{aq}^- during the laser pulse, reaction 11, and, as in the case of Ac-Phe, the



$$\mathrm{NH_2\underset{CH_2Ph}{\underset{|}{C}}HCONH_2} \xrightarrow{h\nu} \begin{cases} \mathrm{PhCH_2\cdot + NH_2\dot{C}HCONH_2} & (10) \\ e_{aq}^- + \mathrm{R}\cdot & (11) \end{cases}$$

growing in of an absorption due to $PhCH_2\cdot$ at a rate $k = 8.0 \pm 1.5 \times 10^5$ sec^{-1}. These results indicate that (a) photoionization is from the triplet state via a biphotonic mechanism, and (b) photodissociation to form $PhCH_2\cdot$ is from the triplet state and is monophotonic in alkaline solutions (when the NH_3^+ group is ionized), and biphotonic[7] at pH 5.1 when the amino group is present as NH_3^+.

Glycylphenylalanylglycine. The laser photolysis of Gly-Phe-Gly ($pK_a^1 \sim 3.6$ and $pK^2 \sim 8$), a dipeptide, in water at pH 5.0 gives immediately after the pulse a transient spectrum with maxima at ~325 and ~245 nm, Figure 7. The spectrum at 10 μsec after the pulse shows the characteristic spectrum of the benzyl radical superimposed on the spectra of other transient species. From the rate of formation of $PhCH_2\cdot$ monitored at 260 nm, the decay rates of the T-T absorption of Gly-Phe-Gly at pH 5.0, 11.1, and 0 were found to be $5.7 \pm 0.8 \times 10^5$, $6.5 \pm 1.5 \times 10^5$, and $5.2 \pm 1.0 \times 10^5$ sec^{-1}, respectively. The triplet is quenched by oxygen, with $k_q = 3.8 \pm 0.5 \times 10^9$ M^{-1} sec^{-1} at pH 5.8; see Table II. In the presence of oxygen, a strong decrease in the radical absorbance at 260 nm is observed.

The hydrated electron produced was formed during the laser pulse with $\phi_{e_{aq}^-}$ of 0.038 and 0.034 at pH 5.0 and 12.5, respectively. It is interesting to note that this yield is essentially independent of pH. This is in agreement with the results obtained for Ac-Phe and Ac-Phe-NH_2, i.e., in all cases where the α-amino group is absent. In the presence of an NH_3^+ group, ionization of this group leads to a reduction in $\phi_{e_{aq}^-}$, as found for Phe and Phe-NH_2. The photoionization of Gly-Phe-Gly occurs presumably via a biphotonic mechanism with the triplet state as precursor, as observed for the other compounds.

The photodissociation process is monophotonic from the triplet state, as can be seen from Figure 7.

Conclusion

The absorption and fluorescence spectra of phenylalkylcarboxylic acids, phenylalanine and related model compounds in water at room temperature have been interpreted[13,14] in terms of intramolecular charge transfer interactions. The carboxylic and amino groups affect the electronic transitions of the aromatic ring even though they are separated from the ring by two methylene groups. The substituents have been classified[13] with regard to their inductive effects on the aromatic ring as $NH_3^+ > COOH > NH_2 > COO^-$. Lowering the ionization potential of the phenyl group by substituents increases the quenching of the fluorescence (i.e., decreases ϕ_F). These charge transfer interactions exhibit an enhancement of phosphorescence yields, low fluorescence yields, an increase in the intersystem crossing process k_{ISC}, and an enhancement in spin-orbit coupling.

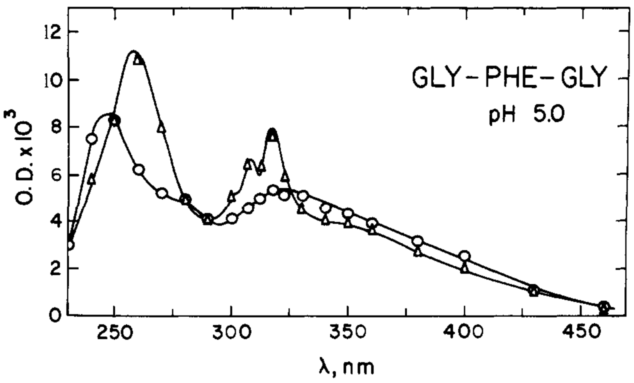


Figure 7. Absorption spectra of the transient species produced on optical excitation at 265 nm of glycylphenylalanylglycine (4.0 mM) at pH 5.0, in the presence of N_2O (1 atm) and 1.0 M t-BuOH. OD were read at 20 nsec (○) and at 10 μsec (△) after the pulse.

From the results presented above, the following conclusions can be made. (1) The photoionization of phenylalanine and related compounds and peptides in water at temperatures 5-70° (the range examined) has the triplet state as the precursor, and electron ejection occurs *in all cases* via a biphotonic process. The biphotonic mechanism prevails whether the amino group is present as NH_3^+ or NH_2. The $\phi_{e_{aq}^-}$, however, is dependent on the state of protonation and decreases when the α-amino group is present as NH_2. The following photophysical processes are suggested for all these compounds.

$$^0\mathrm{S(Phe)} \xrightarrow{h\nu_1} {}^1\mathrm{S(Phe)}$$

$$^1\mathrm{S(Phe)} \to \begin{cases} ^0\mathrm{S(Phe)} + h\nu_F \\ ^0\mathrm{S(Phe)} \end{cases}$$

$$^1\mathrm{S(Phe)} \longrightarrow {}^1\mathrm{T(Phe)}$$

$$^1\mathrm{T(Phe)} \longrightarrow {}^0\mathrm{S(Phe)}$$

$$^1\mathrm{T(Phe)} \longrightarrow {}^0\mathrm{S(Phe)} + h\nu_P$$

$$^1\mathrm{T(Phe)} \xrightarrow{h\nu_2} {}^*\mathrm{T(Phe)}$$

$$^*\mathrm{T(Phe)} \to \begin{cases} \text{electron ejection} \\ \text{dissociation} \end{cases}$$

Some photochemistry from the ^{1}S(Phe), ^{1}T(Phe), and *T(Phe) states presumably occurs as well.

(2) The triplet states of these compounds have T-T absorptions in the uv region with maxima at ~310 and ~240 nm, and lifetimes of ~1-3 μsec (Table I). The presence of an amino group somewhat reduces the lifetime, e.g., for Phe at pH 7.5 $\tau = 3.1$ μsec, whereas for β-phenylpropionic acid at pH 7.3, $\tau = 5.0$ μsec. The replacement of the amino group in Phe by a peptide linkage, e.g., in N-Ac-Phe and Gly-Phe-Gly, also reduces the lifetime. This is suggested to be due, in part, to intramolecular quenching by the -CONH- linkage (see also ref 9).

(3) At all pH values the observed photodissociation reactions occur via the triplet state as precursor. The dissociation processes are biphotonic when the substituents are present as ($COOH$, NH_3^+), (COO^-, NH_3^+), and

($CONH_2$, NH_3^+) and are monophotonic when present as (COO^-, NH_2), (COO^-, -CONH-), (-CONH-, -CO-NH-), and ($CONH_2$, NH_2).

(4) It follows from (3) above that phenylalanine present in protein should dissociate rapidly, in competition with any transfer of energy from ^{3}Phe to other aromatic compounds and functional groups. Hence, contrary to earlier views,[1,2] direct optical excitation of Phe in proteins may be expected to lead to some dissociation reactions. Optical excitation of proteins by high intensity light sources (e.g., flash, laser, or nuclear detonation) will, in addition, lead to the photoionization of phenylalanine.

(5) The photoionization of benzene in water is reported for the first time. Its $\phi_{e_{aq}^-}$ of 0.024 is lower than that of toluene (0.041) and phenylalanine (0.034) at pH 7.0. The photoionization of pyrazine (which is isoelectronic with benzene) in water, under similar laser photolysis conditions, has also been observed[29] with a very low quantum yield.

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Excited State Chemistry of Aromatic Amino Acids and Related Peptides. III. Tryptophan

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Abstract: Using single pulses of ~3.6-15 nsec duration from a frequency quadrupled neodymium laser emitting at 265 nm, and the technique of kinetic absorption spectrophotometry, a detailed study of the photophysics and photochemistry of indole, tryptophan, and peptide derivatives in water has been carried out. The following compounds were examined: indole, indole-3-propionic acid, *N*-methylindole, tryptophan, tryptamine, *N*-methyltryptophan, *N*-acetyltryptophan, tryptophanylglycine, and glycyltryptophanylglycine. The triplet-triplet absorption spectra and lifetimes of the triplet states of these compounds were determined. The T-T absorption maximum is at 450 ± 10 nm in all cases. The lifetimes in neutral solution range from ~11 to 16 μsec. With Trp, tryptamine, and Try-Gly, shorter lived transients (T_1) with λ_{max} ~450 nm and τ ~20-45 nsec are observed in addition. The T_1 transients are tentatively suggested to be triplet states. They are not precursors of the longer lived triplet states, and are observed only when a terminal NH_3^+ group is present in the molecule. T_1 is not observed at pH 10 in Trp. The triplet states are effectively quenched by oxygen ($k_q \sim 5 \times 10^9\ M^{-1}\ sec^{-1}$) and by disulfides RSSR ($k_q \sim 4\text{-}6 \times 10^9\ M^{-1}\ sec^{-1}$). The quenching mechanism with RSSR compounds is shown to occur via an electron transfer process, with the formation of the $RSSR\cdot^-$ radical anion. The photoionization of indoles and tryptophan derivatives is found to occur with a relatively high quantum yield. In neutral solution, e_{aq}^- with λ_{max} ~720 nm and the cation radical with λ_{max} ~550 nm are observed. The cation radical has a lifetime of ~10^{-6} sec and decays to give the neutral indole radical. In alkaline solutions it is shorter lived due to reaction with OH^- ions, while in acid solutions the cation radical has a lifetime of a few hundred microseconds. The dependence upon pH, temperature, and 265-nm light intensity of the yields of the triplet states and the photoionization processes were examined. It is concluded that the photoionization of the indoles occurs, under the conditions studied, via a predominantly monophotonic process from a higher excited singlet state and/or a vibrationally excited lowest singlet state. With Trp, ~10% of the e_{aq}^- produced are formed from the decay of the lowest excited singlet state. These results are compared with those of a similar study with tyrosine and phenylalanine. The possible implications of the photoionization of Trp in proteins are discussed.

The interaction of the tryptophan chromophore with radiant energy has been extensively studied[1,2] with the object of obtaining information on the effects of the physical environment, particularly as pertains to its presence in proteins. In the excited state, chromophores are generally more reactive than in the ground state and thus physical and chemical perturbations usually have a greater influence on both the fluorescence emission and its properties. The quantum yield