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

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*Contribution from the Pioneering Research Laboratory, U.S. Army Natick Laboratories, Natick, Massachusetts 01760. Received August 9, 1974*

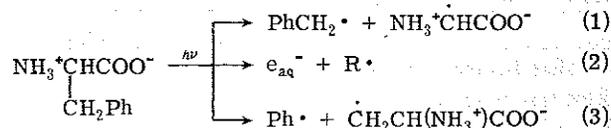
**Abstract:** A pulsed frequency quadrupled neodymium laser emitting at 265 nm (~15 nsec duration) was used to optically excite phenylalanine and derivatives in water at 25°. The following systems were studied: benzene, toluene, phenylpropionic acid, phenylalanine, *N*-acetylphenylalanine amide, phenylalanine amide, and glycyphenylalanylglycine. 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  $\text{NH}_3^+$  group, and decreases when the  $\alpha$ -amino group is present as  $\text{NH}_2$ . 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  $\rightarrow$  tyrosine  $\rightarrow$  tryptophan was feasible and probable in protein macromolecules containing these aromatic amino acids.<sup>1,2</sup> However, the destruction of phenylalanine (Phe) occurs on uv irradiation of proteins.<sup>3</sup> 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<sup>4-6</sup> are  $\phi_F = 0.025$  and  $\tau_F = 6.8$  nsec. A marked temperature dependence was found,<sup>5</sup> with  $\phi_F$  and  $\tau_F$  decreasing with increasing temperature in the range 2–68°. This deactivation process was indicated<sup>3</sup> to be due mainly to internal conversion and only to a small extent due to intersystem crossing. The  $\phi_F$  decreases<sup>4</sup> by ~30% on ionization of the COOH group and ~15% on ionization of the  $\text{NH}_3^+$  group. The phosphorescence of Phe has been observed<sup>1</sup> only in glasses at 77°K and has a band maximum at 385 nm and  $\tau_P \sim 5.5$  sec.

Flash photolysis studies<sup>7</sup> 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 de-

pendent on the state of ionization of the free end groups COOH and  $\text{NH}_3^+$ , 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- $\text{NH}_2$ , and *N*-Ac-Phe were biphotonic, whereas in alkaline solutions (above the  $pK_a$  of  $\text{NH}_3^+$ ) only one quantum was required to bring about the same photodissociation reaction; (e) the triplet state was also involved in alkaline solutions.



The triplet state of Phe and related compounds was not observed,<sup>7</sup> 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  $\beta$ -phenylpropionic acid, toluene, Phe, *N*-Ac-Phe, *N*-Ac-Phe- $\text{NH}_2$ , Phe- $\text{NH}_2$ , and Gly-Phe-Gly were observed and their lifetimes were determined. The pH and temperature dependen-

ces of the photoionization and photodissociation processes were examined, and the mechanisms leading to these dissociations are discussed in detail. Preliminary results have been presented.<sup>8</sup>

### Experimental Section

Details of the experimental set-up and conditions used have been given elsewhere.<sup>9</sup> 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 M phosphates, and 1.0 M borate buffers. Fresh samples were used for each laser pulse.

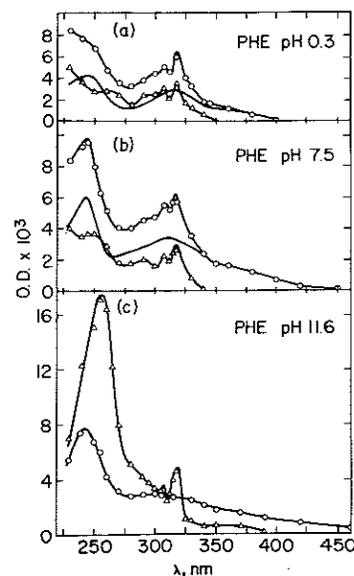
Actinometry was based on anthracene in cyclohexane solutions. The T-T absorption of anthracene was monitored at 428 nm (using narrow slits on the monochromator),  $\epsilon_{428} = 6.47 \times 10^4 M^{-1} \text{ cm}^{-1}$  (from ref. 10) and a  $\phi_{ISC} = 0.75$  (from ref 11).

### 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  $\epsilon_{258} \sim 190 M^{-1} \text{ cm}^{-1}$ . Changes in the spectrum of phenylalanine with pH have been observed<sup>12</sup> indicative<sup>13,14</sup> 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 ( $pK_a^1 = 1.83$  and  $pK_a^2 = 9.13$ ) 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  $\lambda_{\text{max}} \sim 720$  nm, assigned to the hydrated electron  $e_{\text{aq}}^-$ , was found but is not shown in Figure 1b. At 10  $\mu\text{sec}$  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  $\mu\text{sec}$  spectrum resembles that previously observed<sup>7</sup> under flash photolytic conditions (time resolution ~10  $\mu\text{sec}$ ) which was assigned primarily to the benzyl and  $\text{NH}_2\text{CHCOO}^-$  radicals (reaction 1). The spectrum of the benzyl radical was recently redetermined,<sup>15</sup> and maxima at 318, 307, and 258 nm were observed, under similar experi-



**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 (O) and 10  $\mu\text{sec}$  ( $\Delta$ ) 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  $\text{N}_2\text{O}$  (1 atm) and 1.0 M *tert*-butyl alcohol to scavenge the optically generated hydrated electrons (see text).

mental conditions, with extinction coefficients of  $9.0 \times 10^3$ ,  $4.7 \times 10^3$ , and  $2.5 \times 10^4 M^{-1} \text{ cm}^{-1}$ , respectively. The glycine radical has a  $\lambda_{\text{max}} \sim 250$  nm and an extinction coefficient which is strongly dependent upon the pH and the acid-base properties of this radical.<sup>16</sup>

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  $k = 3.2 \pm 0.3 \times 10^5 \text{ sec}^{-1}$  (see Table I); (b) the T-T spectrum of Phe is quenched by oxygen with  $k_q = 3.3 \pm 0.3 \times 10^9 M^{-1} \text{ sec}^{-1}$  (see Table II); (c) similar T-T spectra and lifetimes have been observed from toluene,  $\beta$ -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  $\phi$  of the photoprocesses 1-3

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

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

<sup>a</sup> 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). <sup>b</sup> 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 <sup>a</sup>	pH	Quencher	$k_q, M^{-1} \text{sec}^{-1}$
Phenylalanine ( $3.8 \times 10^{-4} M$ )	7.5	O <sub>2</sub>	$3.3 \pm 0.3 \times 10^9$
Phenylalanine ( $3.4 \times 10^{-4} M$ )	11.6	O <sub>2</sub>	$4.5 \pm 1.0 \times 10^9$
Phenylalanine ( $3.8 \times 10^{-4} M$ )	7.5	Ni <sup>2+</sup>	$4.1 \pm 0.2 \times 10^7$
Phenylalanine ( $0.1-2 \times 10^{-2} M$ )	7.5	Phe	$<2.0 \times 10^6$
<i>N</i> -Acetylphenylalanine (3.8 mM)	8.1	O <sub>2</sub>	$3.9 \pm 0.5 \times 10^9$
Glycylphenylalanylglycine (2.5 mM)	5.8	O <sub>2</sub>	$3.8 \pm 0.5 \times 10^9$

<sup>a</sup> 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<sub>2</sub>· 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<sub>2</sub>· 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<sub>2</sub>· 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<sub>a</sub> values of ground state phenylalanine. The initial T-T titration curve also follows very closely the fluorescence curve<sup>4,13</sup> for Phe. The lifetime of <sup>3</sup>Phe was found to be only slightly dependent upon pH with  $\tau = 2.4, 3.1,$  and  $2.0 \mu\text{sec}$  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<sub>a</sub><sup>2</sup> = 9.13. Due to reaction 4,  $k_4 = 2.3 \times 10^{10} M^{-1} \text{sec}^{-1}$  (ref 17), it



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 <sup>3</sup>Phe (see below), it follows that  $\phi_{e_{aq}^-}$  will reflect the  $\phi_{T-T}$ .

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<sub>a</sub> 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<sub>2</sub>· radicals (i.e., the photodissociation process 1) and, only in small part, the change in the extinction coefficients of the acid-base forms<sup>18</sup> of the additional radicals produced. Such an increase in radical yield with pH was also reported<sup>7</sup> in the

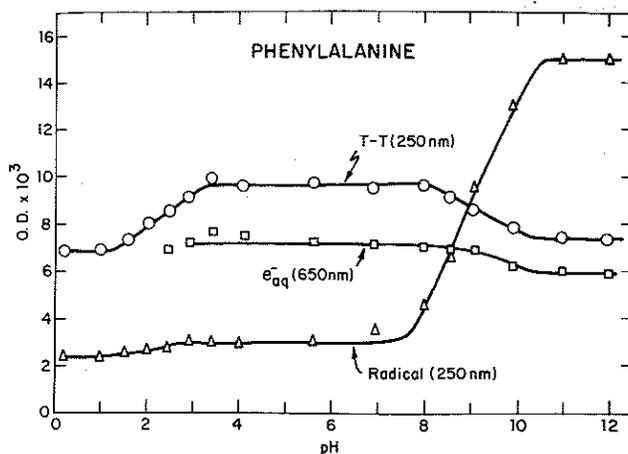


Figure 2. Titration curves of the transient species produced on optical excitation of phenylalanine (4.0 mM) at 25°. OD measured at 20 nsec (O, □) and at 10 μsec (Δ) after the laser pulse.

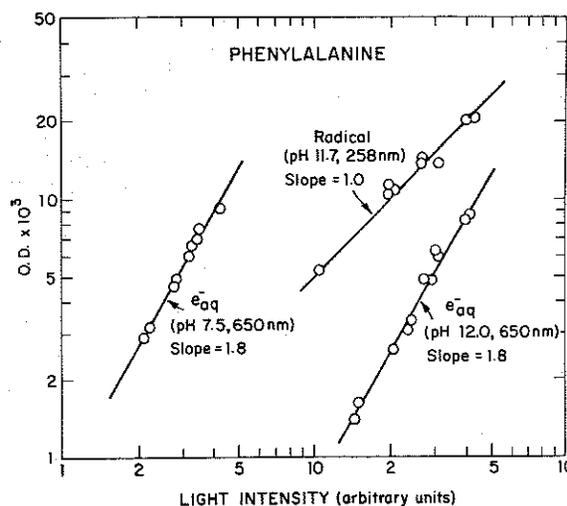


Figure 3. Dependence upon 265 nm light intensity (using a 15 nsec laser pulse) of the yields of transient species produced from phenylalanine (4.0 mM, 25°) in water at pH 7.5, 11.7, and 12.0.

flash photolysis of Phe.

**Dependence of Yields upon Light Intensity.** The flash photolysis study<sup>7</sup> 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<sub>a</sub> = 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<sup>7</sup> that parallel photolytic processes give rise to dissociation and ionization reactions.

**Dependence of Yields upon Temperature.** Fluorescence studies<sup>5</sup> on Phe in neutral aqueous solutions showed a marked decrease in quantum yield with increasing temperature, from  $\phi_F = 0.04$  at 2° to  $\phi_F = 0.008$  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.<sup>17</sup> Hence,  $\phi_{e_{aq}^-}$  decreases more sharply with increasing temperature than indicated in Figure 4.

**Excited State Chemistry of Phenylalanine.** From the laser

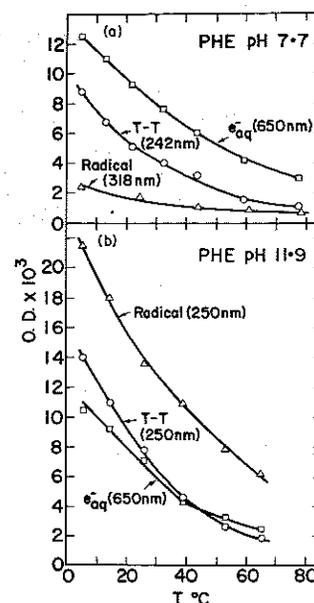
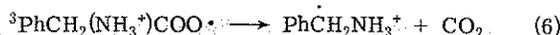
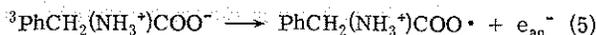
**Table III.** Relative Yields of Photoionization and Photodissociation Processes of Phenylalanine and Related Compounds Optically Excited at 265 nm in Water at 25<sup>o</sup>a

Substrate	pH	$e_{aq}^-$		Ratio OD <sub>240</sub> <sup>b,c</sup>	Ratio OD <sub>318</sub> <sup>b,d</sup>
		$\phi_{e_{aq}^-} \times 10^2$	Ratio <sup>b</sup>		
Phenylalanine	7.5	3.4	1.0	1.0	1.0
	11.1	2.8	0.8 <sub>2</sub>	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		
<i>N</i> -Acetylphenylalanine	7.5, 11.2	3.6	1.0 <sub>6</sub>	1.2	2.6
<i>N</i> -Ac-Phe-NH <sub>2</sub>	7.5, 10.5	3.2	0.9 <sub>4</sub>	0.9	2.0
Phenylalanineamide	5.2	3.2	0.9 <sub>4</sub>	0.9	0.6
Gly-Phe-Gly	12.5	2.7	0.8	0.8	1.9
	5.0	3.8	1.1	0.9	1.8
	12.5	3.4	1.0	1.3	2.6

<sup>a</sup> Determined from solutions whose absorbance at 265 nm was  $0.50 \pm 0.05$ . <sup>b</sup> Ratio based on Phe at pH 7.5,  $\phi = 1.0$ . <sup>c</sup> OD at  $\sim 20$  nsec after the laser pulse. <sup>d</sup> OD read after the decay of the triplet at  $\sim 10$   $\mu$ sec 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  $\sim 2-3$   $\mu$ sec and its quenching rate constant by ground state Phe is  $< 2.0 \times 10^6 M^{-1} sec^{-1}$ . Toluene and  $\beta$ -phenylpropionic acid in water at pH  $\sim 7.0$  also have triplet lifetimes of a few microseconds. It is interesting to point out that <sup>3</sup>Tyr has<sup>9</sup> a  $\tau = 10$   $\mu$ sec in water at pH 6.0. The quenching rate constant by O<sub>2</sub> is close to diffusion controlled, and similar to that found for <sup>3</sup>Tyr (ref 9) and <sup>3</sup>Trp (ref 19). Contrary to what was suggested,<sup>7</sup> the quenching of <sup>3</sup>Phe by Ni(ClO<sub>4</sub>) is relatively slow,  $k_q = 4.1 \pm 0.2 \times 10^7 M^{-1} sec^{-1}$ . Hence the observed<sup>7</sup> decrease in PhCH<sub>2</sub>· radicals by Ni<sup>2+</sup> is probably due to a fast reaction ( $k \geq 10^9 M^{-1} sec^{-1}$ ) between PhCH<sub>2</sub>· and Ni<sup>2+</sup> ions, and not to the quenching of the triplet state precursor of PhCH<sub>2</sub>· 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<sub>2</sub>· within the duration of the laser pulse (15 nsec) and the photoflash<sup>7</sup> ( $\sim 10$   $\mu$ sec) 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 <sup>3</sup>Phe. The rate of decay of <sup>3</sup>Phe is identical to the rate of formation of PhCH<sub>2</sub>·. 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<sup>7</sup> that the ejected electron came from the COO<sup>-</sup> group in Phe as a result of energy transfer to the carboxyl group

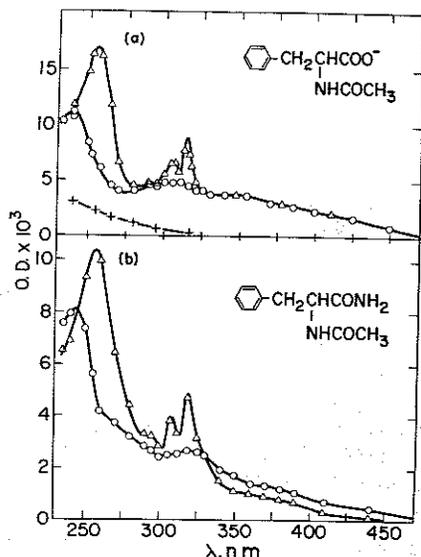


**Figure 4.** Dependence upon temperature of the yields of the transient species produced from optical excitation of phenylalanine in water at (a) pH 7.7, 4.0 mM, and (b) at pH 11.9, 4.0 mM. OD were measured at 20 nsec (O, □) and at 10  $\mu$ sec ( $\Delta$ ) after the laser pulse.

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  $\phi_F$  of benzene at 25<sup>o</sup> has been reported to be 0.005<sup>5</sup> and 0.006,<sup>20</sup> and  $\phi_T = 0.07$ .<sup>21</sup> The triplet-triplet absorption of benzene in water was not observed on laser excitation at 265 nm. The photoionization of benzene ( $\sim 2 \times 10^{-2} M$ ) in water at pH 7.0 was, however, observed and the  $\phi_{e_{aq}^-} = 0.024$  (Table III).

Laser photolysis of toluene (3.0 mM) 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  $e_{aq}^-$ ,<sup>7,22</sup> and PhCH<sub>2</sub>·<sup>23</sup> radicals have previously been reported in flash photolysis studies. The  $\phi_F$  for toluene is 0.13 in ethanol<sup>13</sup> and  $\phi_T = 0.52$  in isooctane<sup>24</sup> 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<sup>25</sup> and

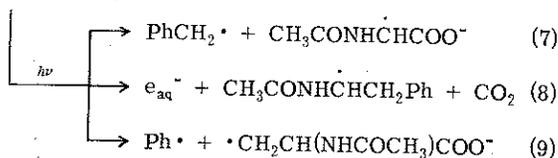
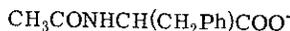


**Figure 5.** Absorption spectra of the transient species produced on optical excitation at 265 nm in water of (a) 4.0 mM *N*-acetylphenylalanine at pH 8.1 and (b) 4.0 mM *N*-acetylphenylalanineamide at pH 8.4. Solutions contained  $N_2O$  (1 atm) and 1.0 M *t*-BuOH (see text). Absorbances were read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

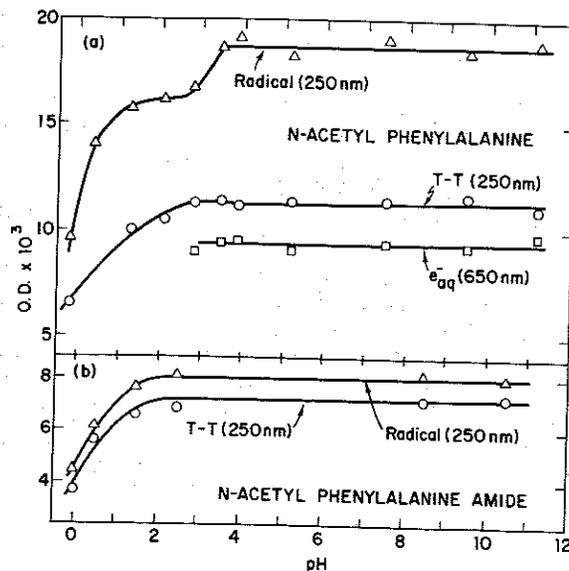
phenolic<sup>26</sup> compounds, it would seem reasonable to assume that photoionization of toluene in water occurs from the triplet state via a biphotonic process.

The flash photolysis of phenylpropionic acid in water was examined.<sup>25</sup> The photodissociation reaction (to produce  $PhCH_2\cdot$  and  $\cdot CH_2COO^-$  radicals) and the photoionization reaction (to form  $e_{aq}^-$ ) were shown<sup>25</sup> 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  $k = 2.0 \pm 0.2 \times 10^5 \text{ sec}^{-1}$  (Table I) and  $\phi_{e_{aq}^-} = 0.034$  (see Table III). The  $\phi_F$  of phenylpropionic acid in ethanol was found<sup>13</sup> to be 0.13.

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



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  $\sim 355$ ,  $\sim 305$ , 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  $\sim 10 \mu$ sec 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  $^3Ac\text{-Phe}$  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  $k = 4.7 \pm 0.4 \times 10^5 \text{ sec}^{-1}$  at pH 8.1 and with a  $k = 9.6 \pm 0.6 \times 10^5 \text{ sec}^{-1}$  at pH 1.4 in 4 mM solutions. In  $O_2$ -saturated solutions a net



**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 (O,  $\square$ ) and at 10  $\mu$ sec ( $\Delta$ ) after the pulse.

decay at 240 nm, due to the quenching of  $^3Ac\text{-Phe}$ , was observed and  $k_q = 3.9 \pm 0.5 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$  was derived at pH 8.1.

In addition to the spectra shown in Figure 5a, the  $e_{aq}^-$  spectrum with  $\lambda_{max} \sim 720 \text{ nm}$  was also observed.

The photoionization process, reaction 8, occurs from the triplet state via a biphotonic mechanism.<sup>7</sup> 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  $\mu$ sec spectrum, is probably associated with the formation of  $e_{aq}^-$ . It was suggested<sup>7</sup> 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.<sup>27</sup>

On photolysis of Ac-Phe in oxygen-saturated solution, a weak spectrum is observed  $\sim 1.0 \mu$ sec 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  $\sim 250 \text{ nm}$  and relatively low extinction coefficients.<sup>28</sup>

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<sup>7</sup> that the ejected electron came from the ionized carboxyl group.



(CONH<sub>2</sub>, NH<sub>3</sub><sup>+</sup>) and are monophotonic when present as (COO<sup>-</sup>, NH<sub>2</sub>), (COO<sup>-</sup>, -CONH-), (-CONH-, -CONH-), and (CONH<sub>2</sub>, NH<sub>2</sub>).

(4) It follows from (3) above that phenylalanine present in protein should dissociate rapidly, in competition with any transfer of energy from <sup>3</sup>Phe to other aromatic compounds and functional groups. Hence, contrary to earlier views,<sup>1,2</sup> 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  $\phi_{\text{eq}}$  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<sup>29</sup> with a very low quantum yield.

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