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## Excited State Chemistry of Aromatic Amino Acids and Related Peptides. I. Tyrosine

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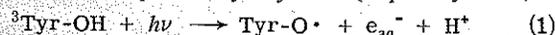
**Abstract:** Optical excitation of phenolic compounds and tyrosine and tyrosyl peptides in water was carried out using a pulsed ( $\sim 15$  nsec duration) frequency quadrupled neodymium laser emitting at 265 nm. The compounds studied include phenol, anisole, *p*-cresol, tyrosine, *p*-hydroxyphenylpropionic acid, tyramine, tyrosylglycine, *N*-acetyltyrosylglycine, glycytyrosylglycine, and cystinylbistyrosine. The lifetimes and triplet-triplet absorption spectra of the triplet states of these compounds were determined. The triplets have  $\tau \sim 3-10$   $\mu$ sec and absorb mainly in the uv region. These triplet states are effectively quenched by oxygen ( $k_q \sim 5 \times 10^9 M^{-1} \text{sec}^{-1}$ ) and by disulfides RSSR ( $k_q \sim 2-4 \times 10^9 M^{-1} \text{sec}^{-1}$ ). The quenching mechanism was established to occur via an electron transfer with the formation of the superoxide radical  $\cdot\text{O}_2^-$  and the RSSR $\cdot^-$  radical anion. A correlation is indicated between the quenching rate constants of  $^3\text{Tyr}$  by organosulfur compounds and the reactivity of these compounds toward  $e_{aq}^-$ . The photoionization of these phenolic compounds and tyrosyl peptides is shown to occur primarily from the triplet state via a biphotonic process. These laser experiments confirm earlier flash photolysis studies from this laboratory. The pH and temperature dependences of the yields of triplets and photoionization products ( $e_{aq}^-$  and phenoxy radicals) have been examined. The implications of these results to the excited state chemistry of tyrosyl containing proteins are discussed.

The macromolecular luminescence of natural proteins is represented largely by the contribution of the luminescence properties of phenylalanine, tyrosine, and tryptophan as perturbed by their presence in a polypeptide chain and the structural influence of the protein. From the studies (see ref 1 and 2 for review) of the fluorescence properties of the aromatic amino acids and proteins at room temperature, and their phosphorescence in organic glasses at low temperature (usually 77°K), various conclusions were reached with regard to energy transfer processes from the singlet excited states and the triplet states of these emitting molecules. The prime interest in these studies was the use of luminescence as a probe for the study of structural properties of proteins. Relatively little work has been carried out on the basic photochemical mechanisms of these systems.

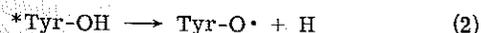
Detailed studies have now been carried out on the chemistry of the excited states of aromatic amino acids, related model compounds, and peptides, with the object of establishing the nature of the precursors leading to the photoionization and photodissociation processes which these molecules undergo. In part I of this series, the particular role of the excited states of phenolic compounds, tyrosine, and tyrosyl peptides will be presented.

The fluorescence quantum yield of phenol and of the zwitterion of tyrosine in aqueous solutions is  $\phi_F \sim 0.21$  at room temperature.<sup>1,2</sup> It is strongly dependent upon temperature,<sup>3</sup> decreasing with increasing *T*. Formation of an amide or a peptide link leads to a reduction in  $\phi_F$ . Fluorescence lifetimes of 3.6 nsec<sup>4</sup> and 5.1 nsec<sup>5</sup> have been reported for tyrosine in water.

The photoionization of phenolic compounds and of tyrosine in water at 25° has been shown<sup>6,7</sup> to occur primarily from the triplet state via a biphotonic process, i.e., the absorption of a second photon by  $^3\text{Tyr-OH}$  (triplet Tyr-OH)



The flash photolysis technique (time resolution  $\sim 10$   $\mu$ sec) was used,<sup>6,7</sup> but the triplet state was not observed. It was also not possible to exclude the possibility that (a)  $\leq 30\%$  of the phenoxy radicals may be produced by rupture of the OH bond, reaction 2, and (b) that a small percentage of the



photoionization process may be occurring by a different mechanism, e.g., from a vibrationally excited singlet state.<sup>8</sup>

Reported below are the results obtained using a frequency quadrupled neodymium laser emitting at 265 nm with pulses of  $\sim 15$  nsec duration. Some preliminary results have been reported elsewhere.<sup>9</sup>

### Experimental Section

A neodymium laser, supplied by Holobeam Inc. (Paramus, N.J.), was frequency quadrupled using temperature-controlled CDA and ADP crystals. The emission at 265 nm could be varied up to  $\sim 25$  mJ/pulse, as read using a Quantronix 504 power meter and Model 500 head. Singles pulses of  $\sim 15$  nsec duration were monitored using an ITT biplanar photodiode 4001 which sampled light at 90° to the laser axis via a "Spectrosil" quartz window beam splitter. Narrow laser pulses of  $\sim 3.5$  nsec duration were obtained using a beam slicer (supplied by Holobeam Inc.) and the intensity was raised back to  $\sim 20-25$  mJ by employing an amplifier (Holobeam) to increase the 1.06  $\mu$  laser output. Unless otherwise stated experiments were performed using pulses of 15 nsec duration. The 265 nm light output typically varied  $\pm 7\%$  within the same day or from day to day. It was routinely monitored for every pulse using the ITT photodiode, and the results were normalized.

The jacketed spectroil quartz optical cell was  $10 \times 10 \times 10$  mm, and the diameter of the laser beam was  $\sim 8$  mm. A Neslab Model RTE-4 was employed as a temperature-controlled circulator.

The 265 nm light intensity was varied by using calibrated Schott filters (No. UG-5). Reproducible results were obtained in this way and this method was used to establish the light intensity dependence of the photophysical and photochemical processes.

Kinetic absorption spectrophotometry of the transients species formed was carried out using either (a) two back-to-back high-intensity Bausch and Lomb monochromators, an EMI 9558QB photomultiplier and associated circuitry<sup>10</sup> (the overall rise time of this circuitry was  $\leq 100$  nsec) or (b) a single high-intensity Bausch and Lomb monochromator, with an RCA IP28 photomultiplier and associated circuitry<sup>11</sup> (the overall rise time of this circuitry was  $\leq 2$  nsec). The signal from the IP28 photomultiplier circuitry was either displayed on a Tektronix oscilloscope, Model No. 7904, with plug-ins 7A19, 7A18, and 7B92, or directly processed using an on-line Biomation 8100 waveform digitizer interfaced to a Hewlett Packard 9830A calculator, programmed for first-order and second-order kinetics.

The monitoring light source was as Osram 250 W Xenon lamp connected to a Kepco KS36-30M power supply. The light output was increased for  $\sim 1$  msec using a booster circuitry,<sup>11</sup> resulting in an increase of  $\sim 400$  times at  $\lambda \leq 280$  nm.

Solutions were prepared just previous to use, and fresh samples were employed for each laser pulse. Highest purity research grade

Table I. Lifetimes of Triplet States of Phenolic Compounds, Tyrosine, and Related Peptides in Water at 25°

Compd <sup>a</sup>	pK <sub>a</sub>	pH	Wavelength monitored, nm	k, sec <sup>-1</sup>	τ
Phenol (3.4 × 10 <sup>-4</sup> M)	10.0	7.5	260	3.0 ± 0.2 × 10 <sup>5</sup>	3.3 μsec
Anisole (5.6 × 10 <sup>-4</sup> M)		8.5	250	3.0 ± 0.2 × 10 <sup>5</sup>	3.3 μsec
<i>p</i> -Cresol (3.5 × 10 <sup>-4</sup> M)	10.2	7.5	260	2.9 ± 0.2 × 10 <sup>5</sup>	3.4 μsec
<i>p</i> -OH phenylpropionic acid (4.4 × 10 <sup>-4</sup> M)	4.6, 10.1	7.5	250	9.9 ± 0.3 × 10 <sup>4</sup> <sup>c</sup>	10.1 μsec
Tyrosine	2.2, 9.1, 10.1	6.0	250	1.8 ± 0.05 × 10 <sup>5</sup> <sup>b</sup>	5.6 μsec
Tyramine (3.4 × 10 <sup>-4</sup> M)	9.5, 10.8	7.5	250	1.0 ± 0.2 × 10 <sup>5</sup> <sup>c</sup>	10.0 μsec
L-Tyrosylglycine (5.0 × 10 <sup>-4</sup> M)		6.0	250	2.9 ± 0.2 × 10 <sup>5</sup>	3.4 μsec
<i>N</i> -Acetyltyrosine (6.0 × 10 <sup>-4</sup> M)		7.5	350	3.1 ± 0.4 × 10 <sup>5</sup>	3.2 μsec
L-Tyrosylglycylglycine (3.0 × 10 <sup>-4</sup> M)		6.0	250	3.2 ± 0.4 × 10 <sup>5</sup>	3.1 μsec
Glycyltyrosylglycine (6.1 × 10 <sup>-4</sup> M)	2.9, 8.5, 10.5	6.0	250	2.7 ± 0.2 × 10 <sup>5</sup>	3.7 μsec
Cystinylbistyrosine (5 × 10 <sup>-4</sup> M)		5.8	250	2.6 ± 0.4 × 10 <sup>7</sup>	38.5 nsec

<sup>a</sup> Numbers in parentheses are the concentration of the substrates at which the lifetimes were determined. <sup>b</sup> Value extrapolated to "zero" tyrosine concentration is 1.0 ± 0.05 × 10<sup>5</sup> sec<sup>-1</sup>. <sup>c</sup> Small second-order component observed.

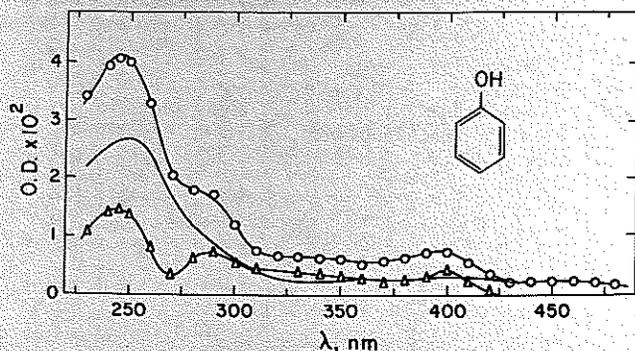


Figure 1. Absorption spectra of the transient species produced on optical excitation at 265 nm of phenol (6.0 × 10<sup>-4</sup> M, pH 7.7, 25°) in water. OD read at 20 nsec (O) and at 15 μsec (Δ) after the 15 nsec laser pulse. The difference spectrum shown by a full line is the T-T absorption spectrum of phenol. At λ > 320 nm, the transient spectra were obtained in the presence of N<sub>2</sub>O (1 atm) and 0.5 M *tert*-butyl alcohol to scavenge the e<sub>aq</sub><sup>-</sup> produced by photoionization (see text).

chemicals were obtained from Calbiochem, Cyclochemicals, Sigma Chemicals, Baker and Adamson, J. T. Baker, and Aldrich Chemicals. Solutions were buffered using perchloric acid, potassium hydroxide, and ~1.0 mM borate and boric acid or 0.2 mM phosphates.

Actinometry was based on anthracene in cyclohexane. The T-T spectrum of anthracene was monitored at 428 nm (using narrow slits), taking <sup>12</sup> ε<sub>428</sub> = 6.47 × 10<sup>4</sup> M<sup>-1</sup> cm<sup>-1</sup> and φ<sub>ISC</sub> = 0.75 (from ref 13).

The absorption spectrum of e<sub>aq</sub><sup>-</sup> produced from the photoionization of the compounds studied is not shown in this work. Its presence was eliminated by saturation of the solution with N<sub>2</sub>O (1 atm). The OH radicals produced from the reaction e<sub>aq</sub><sup>-</sup> + N<sub>2</sub>O → OH + N<sub>2</sub> + OH<sup>-</sup>, were scavenged by ~0.1–1.0 M *t*-BuOH. It was established in all cases that under the conditions of these experiments the N<sub>2</sub>O, *t*-BuOH, and the β-hydroxy radical<sup>14</sup> produced from the alcohol did not interfere with the transient spectra or with the triplet lifetimes and quenching constants reported below.

The rate constants for quenching of the triplet states were derived from linear plots using three to five different concentrations of the quencher in each experiment.

## Results and Discussion

### Phenolic Compounds

**Phenol.** The fluorescence quantum yield of phenol in water is 0.21 and τ<sub>F</sub> ~ 2.1 nsec (see ref 1, 2, and 15). The triplet-triplet absorption spectrum and lifetime of <sup>3</sup>PhOH, like those of tyrosine and other phenolic compounds, have not been measured. The triplet energy level of phenol is reported<sup>15</sup> to be ~75–80 kcal/mol. The flash photolysis of phenol in water showed<sup>7</sup> the characteristic absorption spectrum of the hydrated electron<sup>16</sup> and the phenoxy radical.<sup>17</sup> The photoionization was found<sup>7</sup> to occur from the triplet state via a biphotonic mechanism.

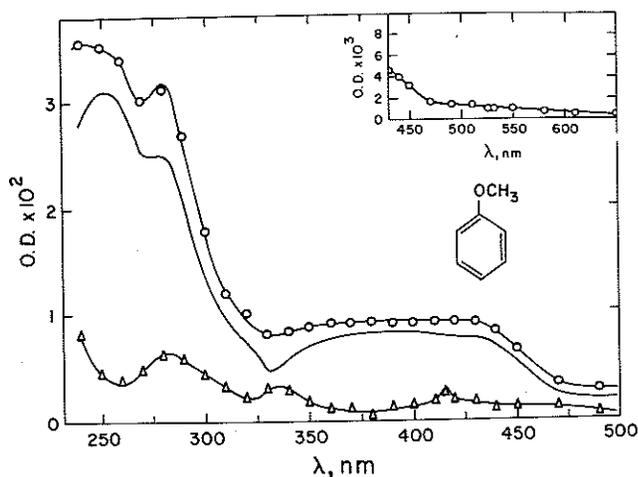
Table II. Rate Constants for Quenching of Triplet State of Tyrosine and Related Peptides in Water at 25°

Tyrosine/peptides	Quencher	pH	k <sub>q</sub> , M <sup>-1</sup> sec <sup>-1</sup> <sup>a</sup>
Tyrosine (6.1 × 10 <sup>-4</sup> M)	O <sub>2</sub>	7.5	4.8 ± 0.2 × 10 <sup>9</sup>
	OH <sup>-</sup>	7–10.3	2.0 ± 0.6 × 10 <sup>10</sup>
	H <sup>+</sup>	2–7	≤ 1.0 × 10 <sup>5</sup>
Tyrosine		7.5	2.5 ± 0.1 × 10 <sup>8</sup>
<i>N</i> -Acetyldiglycine		6.5	1.0 ± 0.4 × 10 <sup>6</sup>
Histidine		3.8	1.6 ± 0.1 × 10 <sup>6</sup>
Histidine		7.7	2.2 ± 0.2 × 10 <sup>6</sup>
Lipoic acid (RSSR)		7.5	4.0 ± 0.2 × 10 <sup>9</sup>
Cystamine (RSSR)		5.2	1.7 ± 0.2 × 10 <sup>9</sup>
Cystamine (RSSR)		7.9	1.5 ± 0.2 × 10 <sup>9</sup>
Dithiodipropionic acid		6.6	1.4 ± 0.1 × 10 <sup>9</sup>
Tyrosine (1.2 × 10 <sup>-3</sup> M)	Tryptophan	7.3	6.0 ± 1.5 × 10 <sup>9</sup>
Phenol (3.4 × 10 <sup>-4</sup> M)	O <sub>2</sub>	7.1	6.1 ± 0.4 × 10 <sup>9</sup>
<i>p</i> -Cresol (3.5 × 10 <sup>-4</sup> M)	Mn <sup>2+</sup>	7.5	1.1 ± 0.1 × 10 <sup>8</sup>
<i>p</i> -Cresol (3.0 × 10 <sup>-2</sup> M)	O <sub>2</sub>	7.5	5.3 ± 0.5 × 10 <sup>9</sup>
<i>p</i> -Cresol (3.0 × 10 <sup>-2</sup> M)	Tryptophan	7.3	6.0 ± 2.0 × 10 <sup>9</sup>
Anisole (5.6 × 10 <sup>-4</sup> M)	O <sub>2</sub>	8.5	6.3 ± 1.0 × 10 <sup>9</sup>
L-Tyrosylglycine (5.0 × 10 <sup>-4</sup> M)	O <sub>2</sub>	6.0	3.6 ± 0.6 × 10 <sup>9</sup>
Glycyltyrosylglycine (6.1 × 10 <sup>-4</sup> M)	O <sub>2</sub>	6.0	3.9 ± 0.3 × 10 <sup>9</sup>
	Lipoic acid (RSSR)	6.0	3.2 ± 0.4 × 10 <sup>9</sup>

<sup>a</sup> Derived from k (sec<sup>-1</sup>) vs. quencher concentration plots.

On optical excitation at 265 nm of phenol in oxygen-free water (6.0 × 10<sup>-4</sup> M, pH 7.7, 25°) using a 15 nsec laser pulse, a transient absorption is observed immediately after the pulse, see Figure 1 (the spectrum of e<sub>aq</sub><sup>-</sup> is not shown). At 15 μsec after the pulse, a different transient spectrum is observed. The bands with maxima at ~400, ~290, and ~245 nm correspond mainly to the characteristic spectrum of the phenoxy radical.<sup>17,18</sup> The difference spectrum (Figure 1) with a relatively strong maximum at ~250 nm, and an absorption extending into the visible region, is suggested to be the T-T absorption spectrum of phenol. Throughout this work in order to obtain the T-T spectra, it has been necessary to subtract an absorption measured after the decay of the triplet from an initial value. This method gives a correct spectrum provided that no new longer lived absorptions are produced during the decay of the triplet. Unless otherwise stated, this is assumed to be the case.

The lifetime of triplet phenol in water (3.4 × 10<sup>-4</sup> M, pH 7.5) is 3.3 μsec; see Table I. This value is not corrected for the quenching of <sup>3</sup>PhOH by ground state phenol (see below). It is quenched by oxygen with k<sub>q</sub> = 6.1 ± 0.4 × 10<sup>9</sup> M<sup>-1</sup> sec<sup>-1</sup> (Table II), obtained using three concentrations



**Figure 2.** Absorption spectra of the transient species produced on optical excitation at 265 nm of anisole ( $5.6 \times 10^{-4} M$ , pH 8.5,  $25^\circ$ ) in water. OD read at 20 nsec (O) and at 15  $\mu$ sec ( $\Delta$ ) after the pulse. The difference spectrum shown by a full line is the T-T absorption spectrum of anisole. At  $\lambda > 320$  nm, the spectra were obtained in  $N_2O$  (1 atm) and 0.5  $M$  *t*-BuOH.

of oxygen. The phenoxy radicals react much more slowly with oxygen,<sup>7</sup> and hence the "base line" on the oscilloscope trace is not affected by the overlapping absorption.

Phenol has a  $pK_a = 9.95$ . On optical excitation of phenolate ions at pH 11–12, both  $e_{aq}^-$  and  $PhO\cdot$  radicals are produced during the 15 nsec laser pulse with a higher quantum yield compared to pH 7.7. No transient spectrum due to  $^3PhO^-$  could, however, be seen (see more below).

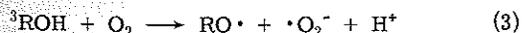
$Mn^{2+}$  ions present as  $Mn(ClO_4)_2$  were found to quench triplet phenol with  $k_q = 1.1 \pm 0.1 \times 10^8 M^{-1} sec^{-1}$ .

**Anisole.** The  $\phi_F = 0.29$  and  $\tau_F = 8.3$  nsec has been reported<sup>19</sup> for anisole in organic solvents. It has a slightly lower triplet energy level than phenol. Figure 2 shows the transient spectra observed at 20 nsec and at 15  $\mu$ sec after the laser pulse. The latter spectrum is presumably due to the  $PhO\cdot$  radical, together with the  $PhOCH_2\cdot$  radical and other species.

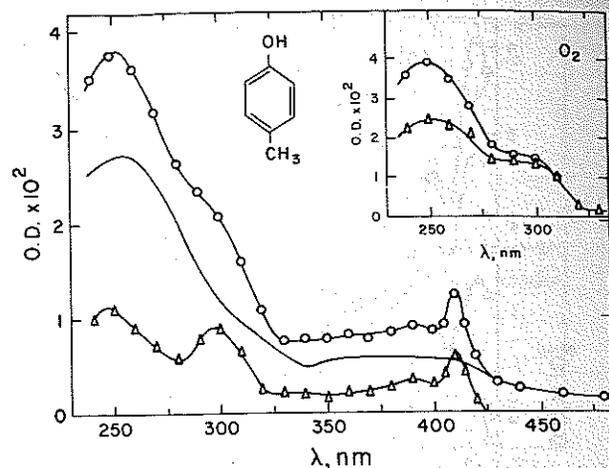
The difference spectrum shown in Figure 2 is suggested to be the T-T absorption of anisole. It is found to decay (in  $5.6 \times 10^{-4} M$  solutions at pH 8.5) with  $k = 3.0 \pm 0.2 \times 10^5 sec^{-1}$ . It is rapidly quenched by oxygen with  $k_q = 6.3 \pm 1.0 \times 10^9 M^{-1} sec^{-1}$ . No changes were observed on increasing the pH from 8.5 to 12.0.

***p*-Cresol.** In cyclohexane solution,<sup>19</sup>  $\phi_F = 0.09$  and  $\tau_F = 2.3$  nsec. Figure 3 shows the transient spectra observed on laser photolysis of *p*-cresol in water at 20 nsec and 15  $\mu$ sec after the pulse. The latter spectrum is probably due mainly to the  $PhO\cdot$  radical, though one cannot exclude the formation of the *p*-OH benzyl radical. The triplet of *p*-cresol, represented by a full line in Figure 3, decays with  $k = 2.9 \pm 0.2 \times 10^5 sec^{-1}$  (in  $3.5 \times 10^{-4} M$  *p*-cresol at pH 7.5).

The  $^3p$ -cresol is quenched by oxygen with  $k_q = 5.3 \pm 0.5 \times 10^9 M^{-1} sec^{-1}$ . The insert in Figure 3 shows the transient spectra observed on photolysis in the presence of  $O_2$ . At 20 nsec after the pulse, the spectrum is essentially the same as that observed in the absence of  $O_2$ . At 1  $\mu$ sec after the pulse (when all the  $^3p$ -cresol are quenched), the spectrum observed is suggested to be due mainly to the phenoxy radical and  $\cdot O_2^-$ .



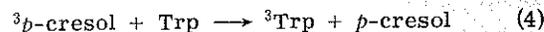
The  $\cdot O_2^-$  radical is known<sup>20</sup> to absorb in the uv region with  $\lambda_{max} \sim 245$  nm and  $\epsilon_{245} = 2 \times 10^3 M^{-1} cm^{-1}$ . As will be discussed below in detail for tyrosine, oxygen can quench the triplet states of phenolic compounds by an electron



**Figure 3.** Absorption spectra of the transient species produced on optical excitation at 265 nm of *p*-cresol ( $6.0 \times 10^{-4} M$ , pH 7.7,  $25^\circ$ ) in water. OD read at 20 nsec (O) and at 15  $\mu$ sec ( $\Delta$ ) after the laser pulse. The difference spectrum shown by a full line is the T-T absorption spectrum of *p*-cresol. At  $\lambda > 320$  nm, the spectra were obtained in  $N_2O$  (1 atm) and 0.5  $M$  *tert*-butyl alcohol (see text). Insert: transient spectra observed on excitation of *p*-cresol in the presence of oxygen (1 atm); OD read at 20 nsec (O) and 1  $\mu$ sec ( $\Delta$ ) after the pulse.

transfer mechanism with the formation of phenoxy and superoxide radicals. This process is monophotonic whereas in the absence of oxygen a second quantum of light is needed to photoionize  $^3ROH$  molecules.

In the presence of tryptophan, triplet-triplet energy transfer was observed from  $^3p$ -cresol. On monitoring the formation kinetics of  $^3Trp$  at 440 nm<sup>21</sup>, a  $k = 6.0 \pm 2.0 \times 10^9 M^{-1} sec^{-1}$  was found (see Table II) for reaction 4. At this wavelength, the absorption of  $^3p$ -cresol is relatively very weak.

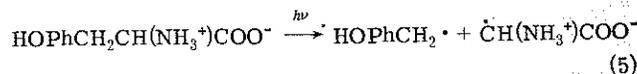


***p*-Hydroxyphenylpropionic Acid.** The  $\phi_F = 0.20$  and the  $\tau_F$  of this compound in water are the same as that of tyrosine in neutral solution.<sup>5</sup> The triplet state lifetime in water is 10.0  $\mu$ sec (Table I), which is longer than that of the tyrosine zwitterion ( $\tau = 5.6$   $\mu$ sec). The protonated  $\alpha$ -amino group would seem to shorten the triplet state lifetime in tyrosine.

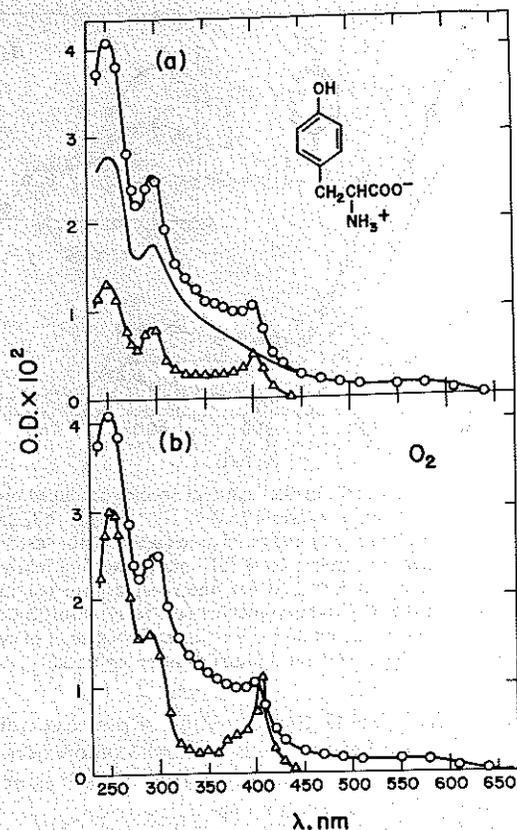
### Tyrosine

**Excited States.** A considerable amount of work has been carried out on the fluorescence of tyrosine in water. The  $\phi_F = 0.21$  and  $\tau_F = 3.6$  nsec for the zwitterion.

On optical excitation at 265 nm of tyrosine ( $pK_a$ 's 2.2, 9.1, 10.1) in oxygen-free aqueous solutions ( $6.0 \times 10^{-4} M$ , pH 7.5,  $25^\circ$ ), somewhat different transient optical spectra are observed when measured at 20 nsec and at 15  $\mu$ sec after the 15 nsec laser pulse, see Figure 4a. The "15  $\mu$ sec spectrum" is suggested to represent primarily the characteristic absorption of the tyrosine phenoxy radical  $Tyr\cdot O$ . It may, in addition, include a weak absorption due to other species, e.g., the *p*-hydroxybenzyl radical. Reaction 5 is suggested on the basis of a similar photodissociation reaction observed on optical excitation of phenylalanine,<sup>22,23</sup> but no evidence for this reaction is presently available.



The difference spectrum shown in Figure 4a is suggested to be the T-T absorption spectrum of tyrosine. Its maxima are at 250, 295, and  $\sim 575$  nm. This spectrum is much broader than the T-T spectrum of phenol or *p*-cresol. The  $^3Tyr$  decays (in  $3.4 \times 10^{-4} M$  solutions at pH 6.0) with  $k$

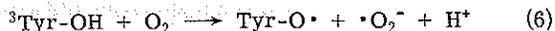


**Figure 4.** Absorption spectra of the transient species produced on optical excitation at 265 nm of tyrosine ( $6.0 \times 10^{-4} M$ , pH 7.5,  $25^\circ$ ) in water. (a) In argon (1 atm), OD read at 20 nsec (O) and at 15  $\mu$ sec ( $\Delta$ ) after the pulse. The difference spectrum shown by a full line is the T-T spectrum of tyrosine. At  $\lambda > 320$  nm,  $N_2O$  (1 atm) and 0.5  $M$  *t*-BuOH were present in the solution. (b) In oxygen (1 atm), OD read at 20 nsec (O) and at 1  $\mu$ sec ( $\Delta$ ) after the pulse.

$= 1.8 \pm 0.5 \times 10^5 \text{ sec}^{-1}$ , see Table I. It is quenched by ground state tyrosine with  $k_q = 2.5 \times 10^8 M^{-1} \text{ sec}^{-1}$  (Table II). At "zero" tyrosine concentration, the triplet state of tyrosine decays with  $k = 1.0 \pm 0.05 \times 10^5 \text{ sec}^{-1}$ .

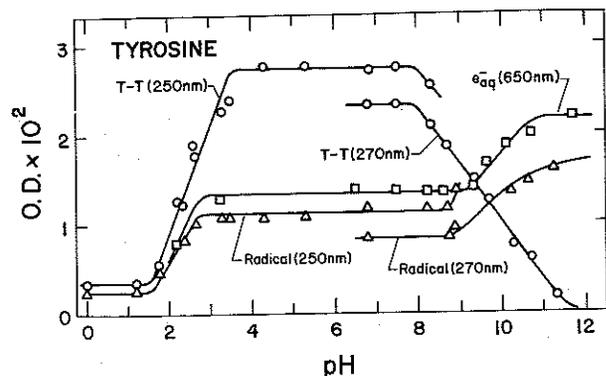
The triplet state of tyramine ( $3.4 \times 10^{-4} M$ , pH 7.5) in water,  $k = 1.0 \pm 0.2 \times 10^5 \text{ sec}^{-1}$ , decays more slowly than  $^3\text{Tyr}$ . Its lifetime is similar to that of *p*-hydroxyphenylpropionic acid (see above).

In the presence of oxygen,  $^3\text{Tyr}$  is rapidly quenched with  $k_q = 4.8 \pm 0.2 \times 10^9 M^{-1} \text{ sec}^{-1}$  (Table II). On optical excitation of  $6.0 \times 10^{-4} M$  tyrosine in the presence of oxygen (1 atm,  $[\text{O}_2] = 1.2 \times 10^{-3} M$ ), the transient spectrum observed at 20 nsec after the pulse is identical to that produced in the absence of oxygen (compare Figures 4a and 4b). At 1.0  $\mu$ sec after the laser pulse, all the tyrosine triplets have decayed and the observed spectrum is essentially similar to the "15  $\mu$ sec spectrum" in the absence of oxygen, but more intense by a factor of  $\sim 2.2$ , see Figure 4b. This increase in the yield of phenoxy radicals is suggested to occur from the quenching of triplet tyrosine by  $\text{O}_2$ .



The superoxide radical has<sup>20</sup> a  $\lambda_{\text{max}} \sim 245$  nm and  $\epsilon_{245} = 2 \times 10^3 M^{-1} \text{ cm}^{-1}$ , and its contribution is mainly to the 255 nm band in Figure 4b. The Tyr-O $\cdot$  radical was found<sup>6</sup> to have an  $\epsilon_{405} = 2.8 \times 10^3 M^{-1} \text{ cm}^{-1}$ .

The Tyr-O $\cdot$  radicals in the absence of oxygen decay by second-order kinetics<sup>6</sup> with  $2k = 1.2 \times 10^9 M^{-1} \text{ sec}^{-1}$ . In the presence of oxygen, they decay by reaction with  $\text{O}_2$  with<sup>7</sup>  $k \leq 10^6 M^{-1} \text{ sec}^{-1}$ .

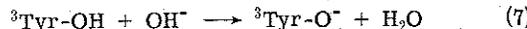


**Figure 5.** Titration curves of the transient species observed on optical excitation of tyrosine ( $6 \times 10^{-4} M$ ,  $25^\circ$ ) in water. The  $e_{\text{aq}}^-$  were read at 20 nsec, T-T at 20 nsec, and the radical at 15  $\mu$ sec after the laser pulse.

**Dependence upon pH.** Figure 5 shows the dependence upon pH of the yields of triplet,  $e_{\text{aq}}^-$ , and radical (mainly phenoxy radicals) produced from the 265 nm excitation of tyrosine ( $6 \times 10^{-4} M$ ,  $25^\circ$ ) in oxygen-free aqueous solutions. The wavelengths monitored in alkaline solutions were changed (as indicated) due to the red shift in the absorption of tyrosinate ions as compared to tyrosine. However, it should be noted that  $\text{OD}_{265}$  remained constant throughout the pH range studied.

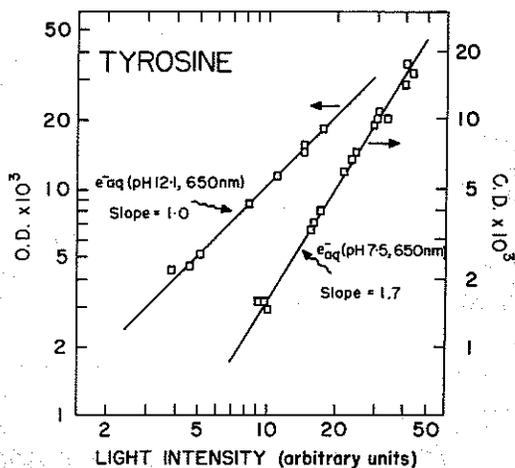
From these titration curves, one can derive in the usual manner approximate dissociation constants. The triplet shows dissociations with  $\text{p}K_a \sim 2.5 \pm 0.2$  and  $9.7 \pm 0.2$ . The  $e_{\text{aq}}^-$  show  $\text{p}K_a$  of  $\leq 2.2$  and  $\sim 10.1 \pm 0.2$  and the radicals  $2.2 \pm 0.2$  and  $10.1 \pm 0.2$ . These values are to be compared with the ground state  $\text{p}K_a$  values of 2.2 (COOH), 9.1 ( $\text{NH}_3^+$ ), and 10.1 (OH) for tyrosine. It is clear that ionization of the phenolic proton and of the carboxyl group play an important role in the formation of triplets,  $e_{\text{aq}}^-$ , and (it follows) phenoxy radicals.

It is important to note the following points: (a) an almost quantitative correlation between the triplet yields and the fluorescence yields<sup>4,5</sup> with pH (both show the same apparent  $\text{p}K_a$  values and their yields decrease to zero in alkaline solutions and decrease to  $\sim 15\%$  in acidic solutions); (b) while the yield of  $^3\text{Tyr}$  decreases in alkaline solution that of  $e_{\text{aq}}^-$  (and Tyr-O $\cdot$ ) increases following the ionization of Tyr-OH; (c) the lifetime of the triplet at pH  $> 8$  is dependent upon  $[\text{OH}^-]$ , with  $k = 2.0 \pm 0.6 \times 10^{10} M^{-1} \text{ sec}^{-1}$  (see Table II), presumably according to reaction 7 (since no



T-T spectrum of  $^3\text{Tyr-O}^-$  could be seen, its lifetime is probably very short,  $\tau < 10^{-9}$  sec; the decrease in the yield of triplets shown in Figure 5 is *not* due to reaction 7 since the absorbances were read at  $\sim 20$  nsec); (d) in view of the apparent very short lifetime of  $^3\text{Tyr-O}^-$ , one cannot exclude the possibility of populating the triplet state from  $^1\text{Tyr-O}^-$ ; however, the fluorescence of  $^1\text{Tyr-O}^-$  has been observed only in low temperature glasses indicating that its lifetimes at  $20^\circ$  is probably  $\leq 10^{10} \text{ sec}^{-1}$ ; (e) at pH below  $\sim 3.5$ , the lifetime of  $^3\text{Tyr}$  is not affected by  $[\text{H}^+]$ , Table II.

**Dependence upon Light Intensity.** Flash photolysis work<sup>7,6</sup> has indicated that the photoionization of tyrosine occurs in neutral solutions from the triplet state via a biphotonic process. Laser photolysis work, Figure 6, supports these conclusions. In alkaline solutions the formation of  $e_{\text{aq}}^-$  from tyrosinate was monophotonic and was suggested<sup>24</sup> to occur from the triplet state. However, since the T-T absorption of  $^3\text{Tyr-O}^-$  was not observed the excited state precursor could be either  $^3\text{Tyr-O}^-$  or  $^1\text{Tyr-O}^-$ . It is not possible at present to establish this point.



**Figure 6.** Dependence upon 265 nm light intensity of the yields of  $e_{aq}^-$  produced on optical excitation of tyrosine ( $6 \times 10^{-4} M$ ) at pH 7.5 and 12.1, 25°.

The slope = 1.7 observed in neutral solution (i.e., less than 2.0, as expected for a process  $\alpha I^2$ ; see Figure 6) may be due to a number of factors, e.g., the formation of  $e_{aq}^-$  via a mechanism other than the absorption of a second quantum of light by  ${}^3\text{Tyr-OH}$ , as discussed above.

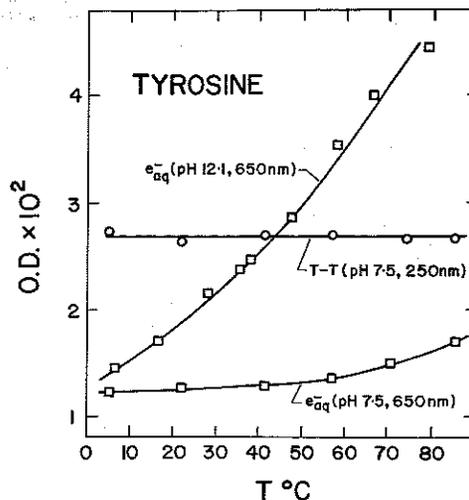
**Dependence upon Temperature.** Over the temperature range 25–95°, the shape of the fluorescence band of tyrosine in water remains unaltered and  $\phi_F$  decreases with increasing temperature.<sup>3</sup> A similar dependence of  $\phi_F$  upon pH was observed<sup>3</sup> at 25 and 95°.

Figure 7 shows the results observed. The  $\phi_T$  was found to be independent of temperature from 5 to 85° at pH 7.5. The  $\phi_{e_{aq}^-}$  from tyrosine (pH 7.5) is somewhat dependent upon temperature, while at pH 12.1  $\phi_{e_{aq}^-}$  is very strongly temperature dependent. It should be pointed out that the absorption maximum of  $e_{aq}^-$  is red shifted<sup>16</sup> with increase in temperature, and the  $\epsilon(e_{aq}^-)$  at 650 nm decreases with increase in  $T$ . These effects are expected to lead to an even more pronounced effect of  $\phi(e_{aq}^-)$  with  $T$ , Figure 7. The following additional points can be made. (a) At pH 7.5, one would not expect<sup>7</sup> a strong dependence of  $\phi_{e_{aq}^-}$  upon temperature for a biphotonic process. The small temperature dependence observed, while the  $\phi_T$  remains unchanged, may be due to either a more efficient biphotonic process or a temperature dependence of  $\phi_{e_{aq}^-}$  produced from another mechanism, e.g., vibrationally excited singlet tyrosine. (b) At pH 12.1, the photoionization reaction is monophotonic. The  $e_{aq}^-$  is produced within the 15 nsec (or 3.5 nsec) laser pulse. The strong dependence of  $\phi_{e_{aq}^-}$  on  $T$  is not inconsistent with a vibrationally excited singlet state as the precursor. (c) With phenylalanine,<sup>23</sup> however, photoionization at pH 7.0 and 12.0 occurs from the triplet state via a biphotonic process, and a strong temperature dependence was observed:  $\phi_{e_{aq}^-}$  decreases with increasing temperature.

**Quenching Reactions of Triplet Tyrosine.** The quenching of  ${}^3\text{Tyr}$  by oxygen and its electron transfer mechanism have been discussed above (see reaction 6). The quenchings by ground state tyrosine,  $\text{OH}^-$ , and  $\text{H}^+$  have also been discussed.

Carbonyl, amide, and peptide groups have been suggested to quench the fluorescence of aromatic amino acids. The lifetimes of the triplet states of the simple peptides of tyrosine (see below), phenylalanine,<sup>23</sup> and tryptophan<sup>21</sup> are all shorter than that of the zwitterions of the corresponding aromatic amino acids. Using *N*-acetylglycylglycine as a model for the peptide linkage, it was found to quench  ${}^3\text{Tyr}$  with  $k_q = 1.0 \pm 0.4 \times 10^6 M^{-1} \text{sec}^{-1}$  (see Table II).

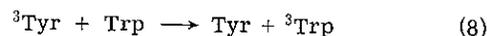
Histidine ( $\text{p}K_a = 1.8, 6.0, 9.2$ ) was found to quench  ${}^3\text{Tyr}$  with  $k_q = 1.6 \pm 0.1 \times 10^8 M^{-1} \text{sec}^{-1}$  and  $2.2 \pm 0.2 \times 10^8$



**Figure 7.** Dependence upon temperature of the yields of transient species produced from the optical excitation of tyrosine ( $6 \times 10^{-4} M$ ) at pH 7.5 and 12.1.

$M^{-1} \text{sec}^{-1}$  at pH 3.8 and 7.7, respectively.

The reaction of tryptophan with  ${}^3\text{Tyr}$  is, of course, an important one in proteins since Trp has a lower triplet energy level. We were able to observe energy transfer from  ${}^3\text{Tyr}$  to Trp. The rate constant  $k = 6.0 \pm 1.5 \times 10^9 M^{-1} \text{sec}^{-1}$  for reaction 8 was determined by monitoring the rate of forma-



tion of  ${}^3\text{Trp}$  at 440 nm.<sup>21</sup> At this wavelength the  ${}^3\text{Tyr}$  absorption is relatively weak compared to  ${}^3\text{Trp}$ .

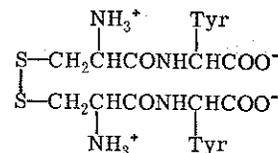
**Quenching by RSSR, RSH, and RSR Substrates.** The quenching of the fluorescence of tyrosine and tyrosyl residues by disulfides and thiols is well documented,<sup>1,2,25-27</sup> but the mechanism is not established.

The quenching of the triplet state of tyrosine by disulfides (RSSR) was recently<sup>24</sup> demonstrated and the mechanism was established to be an electron transfer to form the  $\text{RSSR}^{\cdot-}$  radical anion



Using the cyclic disulfide lipoic acid, the characteristic<sup>28</sup> transient absorption spectrum of the lipoate $^{\cdot-}$  radical anion with  $\lambda_{\text{max}} \sim 420 \text{ nm}$  was observed.<sup>24</sup> The rate constants of reaction 9 for various disulfides were determined, see Table II. Values ranging from  $4.0 \times 10^9$  to  $1.4 \times 10^9 M^{-1} \text{sec}^{-1}$  for lipoate ions and dithiodipropionic acid, respectively, were derived. The formation of  $\text{RSSR}^{\cdot-}$  radicals was confirmed.

The tyrosine in L-cystinylbis-L-tyrosine has a  $\phi_F = 0.016$ <sup>25</sup> in water compared to 0.21 for tyrosine. This loss of



fluorescence is ascribed to "internal" quenching by the  $-\text{SS}-$  group. On optical excitation of this compound at 265 nm, a very short lived weakly absorbing intermediate was observed which decayed with  $k = 2.6 \pm 0.4 \times 10^7 \text{sec}^{-1}$  (Table II). This is ascribed to the triplet state of cystinylbityrosine. Subsequent to the decay of this triplet, an absorption with  $\lambda_{\text{max}} \sim 420 \text{ nm}$  characteristic of the  $\text{RSSR}^{\cdot-}$  radical was observed. The lifetime of this triplet state is  $\sim 100$  times shorter than that of  ${}^3\text{Tyr}$ . It is clear that quenching by the  $-\text{CONH}-$  linkages does not account for the  $\tau \sim 38 \text{ nsec}$  (see tyrosine peptides below), and therefore

Table III. Quenching of the Triplet State of Tyrosine by Organosulfur Compounds in Water at 25°C<sup>a</sup>

Quencher <sup>b</sup>	pH	Ionic form	$k_q, M^{-1} \text{sec}^{-1c}$	$k(e_{aq}^- + Q), M^{-1} \text{sec}^{-1d}$
Cysteamine (8.6, 10.7)	5.2	HSCH <sub>2</sub> CH <sub>2</sub> NH <sub>3</sub> <sup>+</sup>	$5.8 \pm 0.7 \times 10^8$	$3.0 \times 10^{10}$
Cysteine (1.8, 8.3, 10.8)	7.0	HSCH <sub>2</sub> CH(NH <sub>3</sub> <sup>+</sup> )COO <sup>-</sup>	$5.2 \pm 0.7 \times 10^8$	$1.3 \times 10^{10}$
<i>N</i> -Acetylcysteine (~2, 9.5)	5.3	HSCH <sub>2</sub> CH <sub>2</sub> (NHCOCH <sub>3</sub> )COO <sup>-</sup>	$4.0 \pm 0.4 \times 10^8$	$5.6 \times 10^9$
<i>S</i> -Methylcysteine (~2, 8.8)	5.5	CH <sub>3</sub> SCH <sub>2</sub> CH(NH <sub>3</sub> <sup>+</sup> )COO <sup>-</sup>	$3.2 \pm 0.2 \times 10^7$	$7.2 \times 10^8$
Djenkolic acid	5.4	CH <sub>2</sub> -(SCH <sub>2</sub> CH(NH <sub>3</sub> <sup>+</sup> )COO <sup>-</sup> ) <sub>2</sub>	$\leq 2.0 \times 10^7$	$1.0 \times 10^{8e}$
Thiodiacetic acid (3.3, 4.5)	6.3	S-(CH <sub>2</sub> COO <sup>-</sup> ) <sub>2</sub>	$6.0 \pm 3.0 \times 10^6$	$8.3 \times 10^7$
$\beta$ -Thiodipropionic acid (~4)	7.2	S-(CH <sub>2</sub> CH <sub>2</sub> COO <sup>-</sup> ) <sub>2</sub>	$6.5 \pm 1.0 \times 10^6$	$5.8 \times 10^7$
Methionine (2.3, 9.2)	5.7	CH <sub>3</sub> SCH <sub>2</sub> CH <sub>2</sub> CH(NH <sub>3</sub> <sup>+</sup> )COO <sup>-</sup>	$5.0 \pm 2.0 \times 10^5$	$4.5 \times 10^7$

<sup>a</sup>  $1.0 \times 10^{-3} M$  concentration of tyrosine used. <sup>b</sup> Numbers in parentheses are the pK<sub>a</sub> values of the quencher. <sup>c</sup> Derived from  $k$  (sec<sup>-1</sup>) vs. quencher concentration plots. <sup>d</sup> From ref 29. <sup>e</sup> From ref 16.

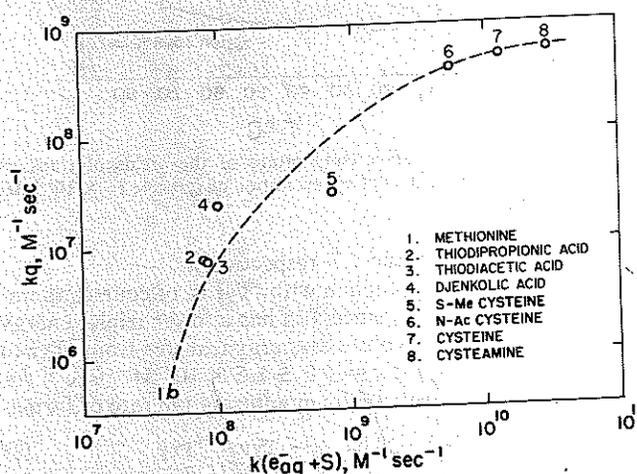
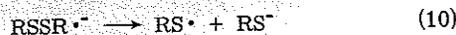


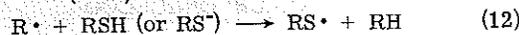
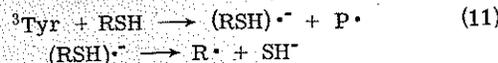
Figure 8. Correlation between the quenching rate constants of the triplet state of tyrosine ( $\sim 1.0 \times 10^{-3} M$ , 25°C) by RSH and RSR compounds and the rate constants of  $e_{aq}^-$  with the same organosulfur compounds (see Table III and text).

the short lifetime is due to internal quenching by the vicinal -SS- group. This finding is considered to be important with respect to the photochemistry of proteins since it is known<sup>28</sup> that the formation of RSSR<sup>-</sup> leads to rupture of the disulfide bridge, and therefore may lead to inactivation of the enzyme.



Sulfhydryl groups and thioethers have also been found to quench <sup>3</sup>Tyr, but these quenching rate constants are well below the diffusion-controlled limits, see Table III. There appears to be a correlation between the quenching rate constants  $k_q$  and the reactivity of the same quenchers toward  $e_{aq}^-$ , as determined<sup>29</sup> by pulse radiolysis, see Figure 8.

It is probably the first such correlation found, and underlines the suggested electron transfer mechanism for the quenching of triplet tyrosine. Reactions 12 and 13 have



been suggested<sup>29</sup> previously. Small yields of RSSR<sup>-</sup> have been found.

The reason for the apparent leveling of  $k_q$  at  $\sim 10^9 M^{-1} \text{sec}^{-1}$  (Figure 8) is not clear at present.

**Tyrosine Peptides.** The photoionization of tyrosyl peptides as indicated by the formation of  $e_{aq}^-$  and phenoxy radicals has been observed in all cases examined.

***N*-Acetyltirosine.** The  $\phi_F$  of this compound<sup>30</sup> is 0.18 at pH 7.0. Optical excitation at 265 nm of *N*-Ac-Tyr ( $6 \times 10^{-4} M$ , pH 7.5) produces, at 20 nsec after the laser pulse, a transient spectrum showing the features of the triplet state, phenoxy radical, and at least one other radical ab-

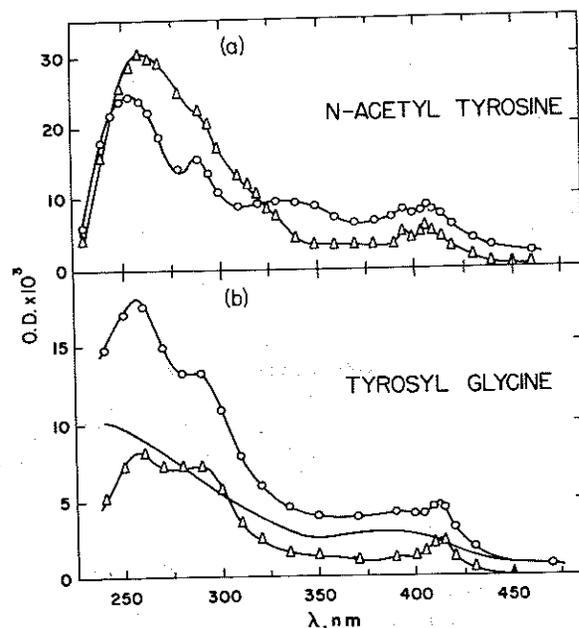


Figure 9. Absorption spectra of the transient species produced on optical excitation at 265 nm in water at 25°C of (a) *N*-acetyltyrosine ( $6 \times 10^{-4} M$ , pH 7.5), and (b) tyrosylglycine ( $6 \times 10^{-4} M$ , pH 6.0). OD read at 20 nsec (O) and at 15  $\mu\text{sec}$  ( $\Delta$ ) after the pulse. The difference spectrum in (b) is the T-T spectrum of Tyr-Gly. At  $\lambda > 320 \text{ nm}$ ,  $\text{N}_2\text{O}$  (1 atm) and 0.5 *M* *t*-BuOH were present in the solution.

sorbing below  $\sim 370 \text{ nm}$ ; see Figure 9a. At  $\sim 15 \mu\text{sec}$  after the pulse, an increase in absorbance is observed at  $\lambda < 320 \text{ nm}$  and a decrease at  $\lambda > 320 \text{ nm}$ . The decrease in absorbance is due to the decay of the triplet state, and a  $k = 3.1 \pm 0.4 \times 10^5 \text{ sec}^{-1}$  was determined (Table I).

The increase in absorbance around 270 nm was also found to have the same rate constant, indicating that it is produced from the decay of <sup>3</sup>*N*-Ac-Tyr. The nature of the species formed, which is presumably a radical(s) produced by a dissociative reaction, is not known. One of the photodissociative reactions produced from <sup>3</sup>Phe was shown<sup>22,23</sup> to lead to the formation of PhCH<sub>2</sub><sup>·</sup> and CH<sub>3</sub>CONHCHCOO<sup>-</sup> radicals. Comparable species may also be formed from *N*-Ac-Tyr and would be consistent with the observed transient spectrum. Similar photodissociative reactions from the triplet state via a monophotonic mechanism are observed whenever the  $\alpha$ -amino group of Tyr is substituted by an acetyl or peptide group; see, e.g., Gly-Tyr-Gly below.

**Tyrosylglycine.** At pH 6.0, the  $\phi_F = 0.07$  for this compound.<sup>30</sup> Laser photolysis of Tyr-Gly ( $6 \times 10^{-4} M$ ) at pH 6.0 shows a transient spectrum whose absorbance decreases between 20 nsec and 15  $\mu\text{sec}$  after the laser pulse, see Figure 9b. The difference spectrum is assigned to the T-T absorption of Tyr-Gly. It decays with  $k = 2.9 \pm 0.2 \times 10^5 \text{ sec}^{-1}$  and is quenched by oxygen with  $k_q = 3.6 \pm 0.6 \times 10^9 M^{-1} \text{sec}^{-1}$  (see Tables I and II).

The spectrum observed at 15  $\mu\text{sec}$  after the pulse shows

**Table IV.** Relative Yields of Intermediate Species Produced from the Optical Excitation at 265 nm of Tyrosine and Related Peptides in Water at 25°

Substrate <sup>a</sup>	pH	$e_{aq}^-$		Initial OD <sub>250</sub> × 10 <sup>2</sup>	Radical OD <sub>250</sub> × 10 <sup>2</sup>
		$\phi_{e_{aq}^-}$ × 10 <sup>2</sup>	Ratio <sup>b</sup>		
Tyrosine	7.5	9.5	1.0	3.8	1.1
	11.5	15.1	1.6		
Tyrosylglycine	6.0	5.1	0.5	1.7	0.8
Glycyltyrosylglycine	6.0	3.5	0.4	1.0	0.7

<sup>a</sup> Determined from solutions whose absorbance at 265 nm was 0.8. <sup>b</sup> Ratio based on Tyr at pH 7.5,  $\phi = 1$ .

the presence of another radical(s) in addition to the characteristic absorption of the phenoxy radical.

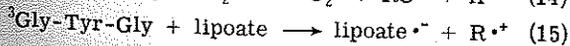
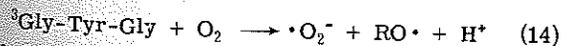
With tyrosylglycine, as with the other tyrosyl peptides examined, the  $e_{aq}^-$  and phenoxy radicals are formed within the 15 nsec laser pulse. As discussed above for tyrosine and other phenolic compounds, the photoionization of tyrosyl peptides is mainly a biphotonic process with the triplet state as the precursor. The photodissociative reactions observed (other reactions are probably occurring but have not been observed by this technique) occur from the decay of the triplet state via a monophotonic mechanism. Hence the latter reactions can occur, e.g., in proteins, even when low intensity light sources are used.

The triplet state of tyrosylglycylglycine was found to decay with  $k = 3.2 \pm 0.4 \times 10^5 \text{ sec}^{-1}$  (Table I).

**Glycyltyrosylglycine.** The quantum yield for photoionization of this peptide is ~40% that of tyrosine (see Table IV). The transient spectra observed at 20 nsec and 15  $\mu\text{sec}$  after the laser pulse are shown in Figure 10. Here again, relatively strong transient absorptions (other than the phenoxy radical) remain after the decay of the triplet, indicative of dissociative reactions. A comparison of the initial and later spectra indicates that these dissociation reactions occur, at least partially, from the triplet state.

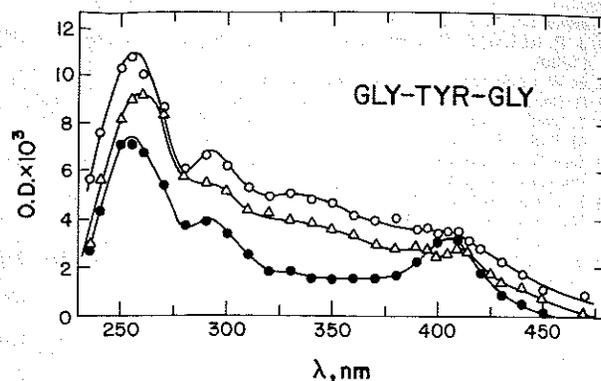
The <sup>3</sup>Gly-Tyr-Gly decays with  $k = 2.7 \pm 0.2 \times 10^5 \text{ sec}^{-1}$  and is quenched by oxygen with  $k_q = 3.9 \pm 0.3 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$  and by lipoate ions with  $k_q = 3.2 \pm 0.4 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$  (see Tables I and II).

On optical excitation of Gly-Tyr-Gly in the presence of oxygen (1 atm), the same transient spectrum as found in O<sub>2</sub>-free solutions is observed at ~20 nsec after the pulse. At ~1  $\mu\text{sec}$  later, all the triplets have been quenched by O<sub>2</sub> and a good fraction of the free radicals have also reacted with O<sub>2</sub> to produce the corresponding peroxy free radicals. The transient spectrum observed (Figure 10) is mainly due to phenoxy radicals (these react slowly with O<sub>2</sub>, see above), O<sub>2</sub><sup>-</sup>,<sup>20</sup> and peroxy radicals.<sup>15,31</sup> Peroxy radicals and O<sub>2</sub><sup>-</sup> usually have  $\lambda_{\text{max}} \leq 250 \text{ nm}$ , and low extinction coefficients compared to phenoxy radicals. As we concluded above for tyrosine, the quenching of <sup>3</sup>Gly-Tyr-Gly by oxygen and by disulfides also occurs via an electron transfer mechanism.



### Conclusions

The lifetimes, absorption spectra, and the chemistry of the triplet states of phenolic compounds, tyrosine, and tyrosine peptides have been studied and determined for the first time. These triplets have lifetimes of ~3–10  $\mu\text{sec}$  in water. They are rapidly quenched by oxygen with  $k_q \sim 5 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$  and by disulfides with  $k_q \sim 2\text{--}4 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$ .



**Figure 10.** Absorption spectra of the transient species produced on optical excitation at 265 nm of glycyltyrosylglycine ( $6 \times 10^{-4} \text{ M}$ , pH 4.5) in water at 25°. (a) In oxygen-free solutions, OD read at 20 nsec (○) and at 15  $\mu\text{sec}$  (△) after the pulse; at  $\lambda > 320 \text{ nm}$ , N<sub>2</sub>O (1 atm) and 0.5 M *t*-BuOH were present in the solution. (b) In oxygen (1 atm), OD read at 1  $\mu\text{sec}$  after the pulse (●).

The quenching of the triplet states of tyrosine and of tyrosyl peptides by O<sub>2</sub> and RSSR is shown to occur via an electron transfer mechanism with the formation of the superoxide  $\cdot\text{O}_2^-$  radical and the RSSR<sup>-</sup> radical anion. The quenching mechanism which affects tyrosyl residues in proteins associated with vicinal disulfide linkages may be explained on the basis of electron transfer processes to the RSSR groups.

The photoionization of tyrosine and tyrosyl peptides in water has been shown to occur mainly from the triplet state via a biphotonic process. The quantum yield of this reaction is, therefore, dependent on the lifetime of the triplet state, the intensity of the exciting light at the appropriate wavelength, and the quantum yield of the biphotonic process. The ionization of the triplet states by O<sub>2</sub> and RSSR is a monophotonic process, and hence could be of great importance in the photochemistry of tyrosyl containing proteins exposed to low intensities of exciting light.

Finally, the  $e_{aq}^-$  produced in the photoionization of tyrosyl proteins can react with aromatic<sup>6,32</sup> and aliphatic<sup>33</sup> amino acids, the peptide linkage,<sup>34</sup> disulfides,<sup>28</sup> and sulfhydryl amino acids<sup>29</sup> and lead to deamination reactions, electron transfer reactions, and ruptures of linkages which may affect the conformation and activity of proteins.

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### References and Notes

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