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

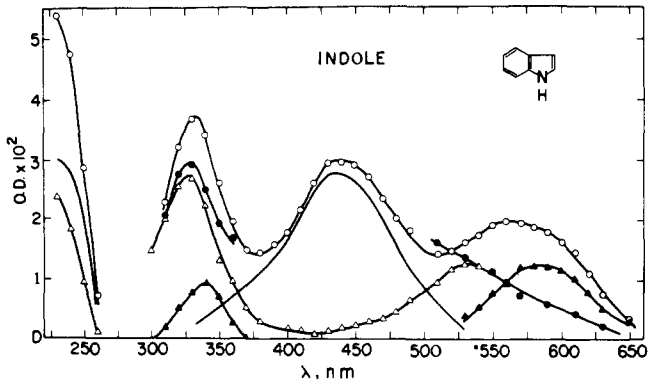


Figure 1. Transient species observed on optical excitation at 265 nm of 1.5×10^{-4} *M* indole in oxygen-free water (pH 7.5, 25°), using a 15 nsec laser pulse. OD read at 50 nsec (○) and 4 μsec (●) after the pulse and at 1 μsec (△) after the pulse in the presence of oxygen (1 atm). The full line represents the difference spectrum and is assigned to the triplet–triplet absorption of indole. The neutral radical is represented by △ and ▲ is obtained from (○ − ●).

of fluorescence of tryptophan (including indole and derivatives and peptides), its quenching, enhancement, and lifetime have been found to be strongly dependent on temperature, pH, nature of the solvent, nature and charge of the quencher, etc.

The radiative and radiationless relaxation processes of the fluorescent state of indole, tryptophan, and related derivatives and peptides have been examined in detail. Fluorescence polarization studies[3] and quantum mechanical calculations [4]for tryptophan indicate that at least two independent overlapping electronic transitions, designated $^1L_a \leftarrow {}^1A$ and $^1L_b \leftarrow {}^1A$, are responsible for the absorption spectra in the 260–310-nm region. The two excited states[5-10] are differentially shifted by solvent and methyl substitution, and could arise from thermally equilibrated ^1L_a and ^1L_b states or from nonrelaxed states. This dual emission has been confirmed for indoles in both polar and nonpolar solvents.[5-10]

For indole, tryptophan, and derivatives in water, the dependence of ϕ_F and τ_F upon pH, temperature, and quenchers has been examined in greater detail.[11-22] For indole at neutral pH and ~25°, ϕ_F values of 0.23,[15,20] 0.28,[18] and 0.4–0.45[12,19,22] have been reported and ϕ_F decreases sharply at pH >10–11. This decrease is associated with the -NH- group in indole. The τ_F at pH ~7.0 is 4.0–4.9[18-20] nsec. For 1-methylindole, $\phi_F = 0.38$[15]–0.46[20] and $\tau_F = 10.3$ nsec.[20] The ϕ_F and τ_F for tryptophan (pK_a = −6.23,[23] 2.38, and 9.39) have been studied by many investigators. At pH ~7.0 ϕ_F values of 0.12,[21] 0.14,[15,18,19] and 0.20,[12] at pH 10–11 (maximum ϕ_F yields) ϕ_F values of 0.36[20] and 0.51,[12] and at pH ≤2.0, ϕ_F values of 0.06[20] and 0.085[12] have been reported. The τ_F at pH ~7.0 are 2.0,[18] 2.6,[13] 2.8,[15,19] 2.9,[14] and 3.0[17] nsec, at pH 10–11 $\tau_F \sim 9.0$ nsec,[17] and at pH ≤2.0 $\tau_F = 2.0$ nsec.[17,20]

The phosphorescence of indole and Trp in low-temperature glasses has been studied;[1,2,24,25] the $^3(\pi,\pi^*)$ indole L_A state has a triplet energy of 2.86 eV.[5] No information is available on the triplet state(s) in fluid solutions.

The photochemistry[27-29] and flash photolysis[30,31] of indole and Trp have been studied. Photoionization was found to be one of the main photoprocesses, but the excited state precursor has not been clearly identified.

This work presents a first study of the laser photolysis of indole, tryptophan, and derivatives in water, employing optical excitation at 265 nm with single pulses of ~3.6–15.0 nsec duration. The triplet states have been observed and their lifetimes determined. The yields of triplets, e_{aq}^-, and radicals have been examined as a function of pH. Preliminary results have appeared elsewhere.[32]

Experimental Section

A frequency quadrupled neodymium laser (Holobeam Inc., N.J.) emitting single pulses of ~3.6 and ~15.0 nsec duration at 265 nm was employed. Full experimental details have been given elsewhere.[33]

Best commercially available research grade chemicals were used and were supplied by Calbiochem, Sigma, Cyclochemicals, and Aldrich. Reagents used were obtained from Baker and Adamson, Mallinckrodt, Eastman, and Aldrich.

Solutions were prepared just prior to use and were buffered using $HClO_4$, H_2SO_4, KOH, phosphates, and borate ($\sim 2\text{–}10 \times 10^{-4}$ *M*). Fresh solutions were used for each laser pulse.

The e_{aq}^- spectrum with λ_{max} ~720 nm produced on optical excitation of indole, Trp, and derivatives was "removed" by performing the experiments in the presence of 1 atm of N_2O and ~0.1–1.0 *M tert*-butyl alcohol (see ref 33). The presence of both N_2O and *t*-BuOH did not interfere with the formation or lifetimes of the triplet states and the radicals observed, under the conditions of our experiments.

Results and Discussion

Indole

In neutral aqueous solution at ~25°, the fluorescence from indole has a τ_F = 4.0–4.9 nsec,[18,20] and ϕ_F values 0.23[15,20] to 0.45.[12,19,22] These ϕ_F values are all much higher than the ϕ_F for Trp in neutral solution (zwitterion form).

On optical excitation at 265 nm of indole (1.5×10^{-4} *M*) in oxygen-free water at pH 7.5 and 25°, a relatively high yield of hydrated electrons is observed, characterized by the transient optical absorption maximum at ~720 nm, in agreement with Grossweiner's results.[34] The total yield of e_{aq}^- is produced during the 15-nsec laser pulse. In addition, the transient spectrum shown in Figure 1 with maxima at ~332, 440, and ~565 nm is observed at ~50 nsec after the laser pulse (e_{aq}^- spectrum not shown; see Experimental Section and ref 33). Additional bands with $\lambda_{max} \leq 230$ nm can also be seen. At 4 μsec after the pulse, the absorbance of the first two bands decrease whereas the 565-nm band is no longer apparent; 30 μsec later the band at 440 nm has decayed and a longer lived absorption with peaks at 330 and 530 nm remains.

On optical excitation of the same indole solution in the presence of oxygen (1 atm = 1.2×10^{-3} *M*), a transient spectrum with maxima at ~328 nm and ~532 nm is observed 1 μsec after the pulse; see Figure 1. This transient absorption is suggested to be that of the neutral indole radical (see below).

The band with a maximum at ~440 nm (Figure 1) is suggested to be ^{3}indole. It decays with $k = 8.6 \pm 0.4 \times 10^4$ sec^{-1} in 1.5×10^{-4} *M* aqueous solution at pH 7.5 (see Table I) and is quenched by oxygen with $k_q = 5.3 \pm 1.0 \times 10^9$ M^{-1} sec^{-1} (see Table II). The quenching rate constant by O_2 is typical of those found for other aromatic *N*-heterocyclic compounds,[35] and for ^{3}tyrosine,[33] ^{3}phenylalanine,[36] and ^{3}tryptophan (see below). The decay of ^{3}indole appears to be independent of indole concentration, up to 10^{-3} *M*.

The initial absorptions at ~565 nm and ~340 nm (Figure 1) are suggested to be due to the indole cation radical $IH^{\cdot+}$, produced from the photoejection of an electron from indole (IH). In neutral solutions it decays with $k \sim 10^6$ sec^{-1}; in alkaline solutions it is shorter lived presumably due to the rapid loss of a proton

$$IH \xrightarrow{h\nu} IH^{\cdot+} + e_{aq}^- \quad (1)$$

$$IH^{\cdot+} \rightleftharpoons I^{\cdot} + H^+ \quad (2)$$

Table I. Lifetimes of Triplet States of Indole, Tryptophan, and Related Peptides in Water at 25°

Compd[a]	pH	k, sec^{-1}[b]	τ
Indole	7.5	$8.6 \pm 0.4 \times 10^4$	11.6 μsec
	1–3 M H_2SO_4	$3.2 \pm 0.3 \times 10^7$	31.2 nsec
Indole-3-propionic acid	7.5	$6.6 \pm 0.6 \times 10^4$	15.2 μsec
N-Methylindole	1.0 M H_2SO_4	$3.0 \pm 0.4 \times 10^7$	33.3 nsec
Tryptophan	7.5	$2.3 \pm 0.2 \times 10^7$[c]	43.5 nsec
	7.5	$7.0 \pm 0.7 \times 10^4$	14.3 μsec
	11.0	$5.0 \pm 1.0 \times 10^4$	20.0 μsec
	2.3	$5.7 \pm 0.5 \times 10^5$	1.8 μsec
	0.2	$3.0 \pm 0.3 \times 10^7$	33.3 nsec
		$6.5 \pm 1.0 \times 10^4$[d]	15.4 μsec
Tryptamine	7.5	$3.0 \pm 1.0 \times 10^7$[c]	33.3 nsec
	7.5	$7.1 \pm 0.7 \times 10^4$	14.1 μsec
N-Methyltryptophan	7.5	$7.5 \pm 0.8 \times 10^4$	13.3 μsec
N-Acetyltryptophan	7.0	$6.1 \pm 0.1 \times 10^4$	16.4 μsec
Tryptophanylglycine	5.0	$5.0 \pm 2.5 \times 10^7$[c]	20.0 nsec
	5.0	$6.2 \pm 0.6 \times 10^4$	16.1 μsec
Glycyltryptophanylglycine	5.0	$8.5 \pm 2.0 \times 10^4$	11.8 μsec

[a] The concentration of the substrates at which the lifetimes were determined was 1.5×10^{-4} M. [b] Decay rate monitored at 440 nm. [c] Short lived intermediate tentatively suggested to be an excited species (see text). [d] In neat methyl alcohol.

Table II. Rate Constants for Quenching of Triplet States of Indole, Tryptophan, and Related Peptides in Water at 25°

Compd[a]	Quencher	pH	k_q, M^{-1} sec^{-1}[b]
Indole	O_2	7.5	$5.3 \pm 1.0 \times 10^9$
Indole-3-propionic acid	O_2	7.5	$4.7 \pm 1.0 \times 10^9$
Tryptophan	O_2	7.5	$5.0 \pm 1.0 \times 10^9$[c]
	Lipoate (RSSR)	7.3	$3.6 \pm 0.4 \times 10^9$[c]
	Anthracene	[d]	$4.0 \pm 0.4 \times 10^9$[c]
Tryptamine	O_2	7.5	$5.7 \pm 1.0 \times 10^9$[c]
N-Methyltryptophan	Lipoate (RSSR)	7.5	$3.1 \pm 0.2 \times 10^9$
Tryptophanylglycine	O_2	5.0	$4.0 \pm 1.0 \times 10^9$[c]
Glycyltryptophanylglycine	O_2	5.0	$4.5 \pm 1.0 \times 10^9$

[a] Solutions contained ~$1–2 \times 10^{-4}$ M concentration of the substrates. [b] Derived from k (sec^{-1}) vs. quencher concentration plots. [c] For T_2 species, see text. [d] In ethyl alcohol.

while in acid solutions (pH 3–4) it has a lifetime of tens of microseconds. Deprotonation of the cation radical $IH\cdot^+$ presumably produces the neutral radical I· with absorption maxima at ~532 nm and ~328 nm. No evidence for the formation of I· from the decay of $IH\cdot^+$ was actually observed, probably due to small differences in their extinction coefficients.

Support for the assignments of the $IH\cdot^+$ and I· radicals can be derived from: (a) the blue shift in the absorption spectrum of the cation radical when compared to the neutral "nitrogen" radical (this is in agreement with the radical spectra of other aromatic N-heterocyclic compounds);[35,37] and (b) the relative stability of the I· radical

$IH\cdot^+$ I·

in the presence of oxygen. Carbon-centered free radicals are known to be very reactive toward O_2 and to produce peroxy radicals with maxima at ≤260 nm[38], and relatively low extinction coefficients. Nitrogen-centered radicals appear to be much less reactive toward oxygen.[37]

Dependence upon pH. The changes with pH of the absorbances of e_{aq}^- (monitored at 650 nm), the indole triplet (440 nm), the cation radical $IH\cdot^+$ (585 and 330 nm), and the neutral radical I· (530 and 330 nm) produced from the optical excitation of indole in water are shown in Figure 2.

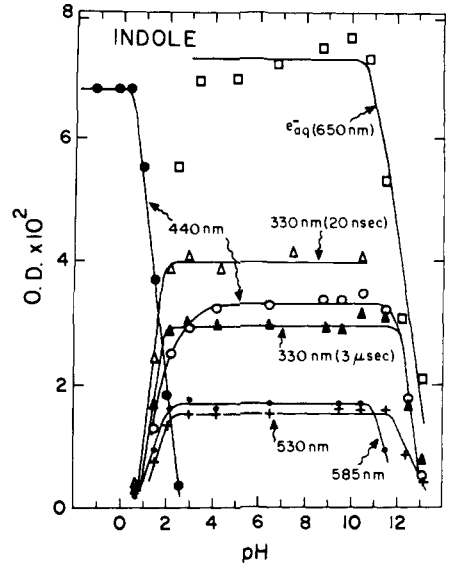


Figure 2. Titration curves of the transient species produced in the laser photolysis of 1.5×10^{-4} M indole in O_2-free water at 25°. OD read at "zero" nsec (●) and at 20 nsec (○, •, □) after the laser pulse. The OD in O_2-saturated solution was read at 1 μsec (+) after the pulse.

In the pH range ~2.5–10.5 no appreciable changes with pH are found. At pH ≥10.5 there is a decrease in the quantum yield of all the transient species monitored. This decrease follows the decrease in ϕ_F of indole in alkaline solution and suggests (a) a decrease in ϕ_{ISC} to form ^{3}indole and (b) that the photoionization of indole has a singlet excited state as the precursor (see more below).

In acid solutions below pH ~2.5, the yields of triplets and radicals decrease with an increase in $[H^+]$. This decrease again follows the decrease in ϕ_F with a decrease in pH. Due to reaction 3, $k_3 = 2.3 \times 10^{10}$ M^{-1} sec^{-1} (ref 39), it was

$$e_{aq}^- + H^+ \longrightarrow H \qquad (3)$$

not possible to monitor $\phi_{e_{aq}^-}$ at pH <3.0.

At pH <2.5 a new very short lived transient absorption was found whose intensity increased with a decrease in pH (Figure 2), with an apparent $pK_a \sim 1.5$. Figure 3 shows the absorption spectrum of this intermediate in 1–3 M H_2SO_4.[40] This species decays with $k = 3.2 \pm 0.3 \times 10^7$ sec^{-1}. The transient is suggested to be a triplet state, $^3IH_2^+$. The apparent $pK \sim 1.5$ may reflect the protonation of $^1IH.$*[41,42] The pK_a of indole to form the cation is ~−6.3, and a $\phi_F = 0.145$ has been reported.[22] It is important to note that on excitation of indole in 1–3 M H_2SO_4 no other transient species were observed (i.e., apparently no photoionization occurred).

Optical excitation of N-methylindole in 1.0 M H_2SO_4 gives a similar transient species as found for indole, which decays with $k = 3.0 \pm 0.4 \times 10^7$ sec^{-1} (see Table I).

Dependence upon Temperature. The ϕ_F of indole in water decreases[19] about five fold between 5 and 50°. This temperature dependence has been suggested[19] to be due to two radiationless deexcitation processes; only one of these is temperature dependent and is associated with electron ejection.

Figure 4 shows the changes in the quantum yields with temperature of the triplet, e_{aq}^-, and the cation and neutral radicals produced from indole in water at pH 7.5. The decrease in ϕ_T with increasing temperature follows qualitatively the same trend as ϕ_F. The yield of photoionization increases markedly with increase in temperature, as observed by Feitelson[26] over a more limited temperature range. This increase in $\phi_{e_{aq}^-}$ is consistent with the conclusion reached

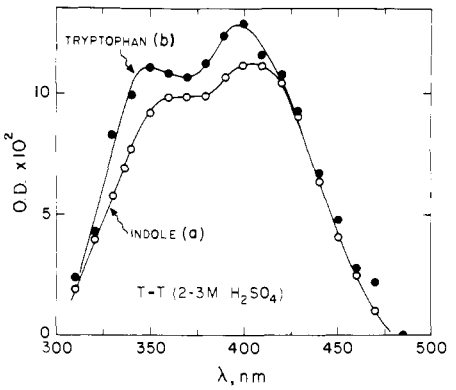


Figure 3. Triplet-triplet absorption spectra observed in acidic solution on laser photolysis in water at 25° of (a) indole (1.5×10^{-4} *M*, in 1–3 *M* H_2SO_4), and (b) tryptophan (1.5×10^{-4} *M*, 0.5–2.0 *M* H_2SO_4). OD read at 0 nsec after the pulse. No other transient species were observed in these solutions.

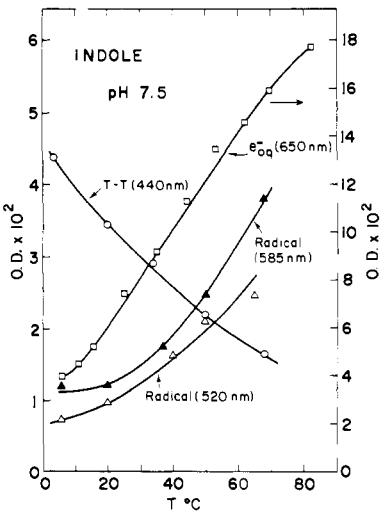


Figure 4. Dependence upon temperature of the yields of transient species produced from the laser photolysis of indole (1.5×10^{-4} *M*) at pH 7.5.

for tryptophan (see below) that the main precursor for the photoionization reaction is a vibrationally excited singlet state. It should be pointed out, however, that ϵ_{650} for e_{aq}^- is temperature dependent (see ref 33 for discussion of this point); its effect would lead to a higher $\phi_{e_{aq}^-}$ with increase in temperature than that shown in Figure 4.

Similar results were obtained on laser photolysis of indole-3-propionic acid at pH 7.5 (see Tables I and II).

Tryptophan

The fluorescence of tryptophan (pK_a = −6.23, 2.38, 9.39) in aqueous solution has been studied in great detail. Some discrepancies still exist in both ϕ_F and τ_F. The "best" value in neutral solution for τ_F is ~2.7–3.0 nsec (see above) which is shorter lived than that of indole. Its lifetime increases to $\tau_F \sim 9$ nsec at pH ~10 and then decreases again with further increase in pH.[17] At pH <3.0, τ_F decreases with an increase in $[H^+]$. The ϕ_F follows the same trend, with "best" values of 0.14 at pH ~7.0, and ~0.42 at pH ~10.0.

On optical excitation of Trp in oxygen-free water at pH 5.4 and 25°, the transient optical spectra observed are shown in Figure 5a. A very short lived species with $\lambda \sim 245$ nm is found with $\tau < 10^{-8}$ sec (at pH 11, $\tau \sim 10$ nsec). This absorption is probably due to the excited singlet-singlet spectrum of tryptophan (zwitterion form). The τ of this singlet decays more slowly at pH ~11 than at pH 7.0, and

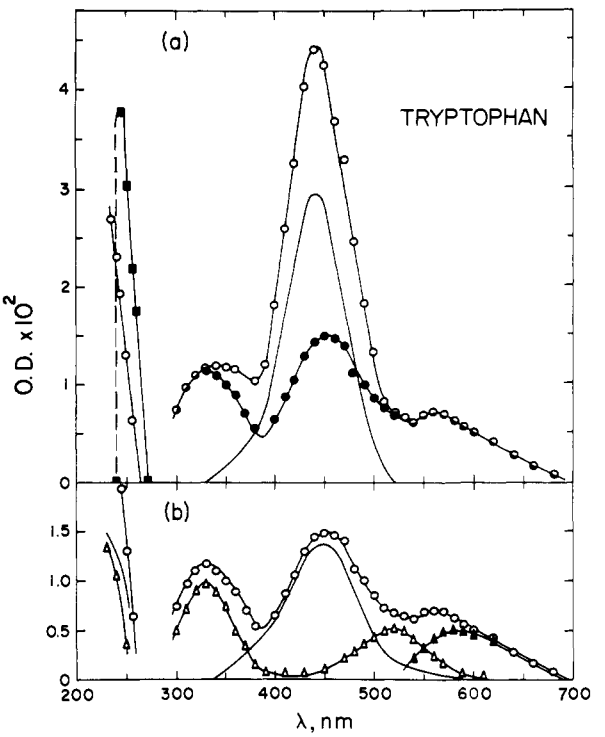


Figure 5. Transient species observed on optical excitation at 265 nm of 1.5×10^{-4} *M* tryptophan in water (pH 5.4, 25°), using a 15 nsec laser pulse. (a) OD read at 0 nsec (○) and 100 nsec (●) after the pulse. The full line represents the difference spectrum referred to as T_1 (see text). The symbols ■ were read at 0 nsec and represent the excited singlet-singlet absorption spectrum of Trp at pH 5.4. (b) OD read at 100 nsec (○) after the pulse in O_2-free solutions, and △ symbols were obtained in the presence of oxygen (1 atm) at 1 μsec after the pulse. The full line represents the difference spectrum and is referred to as T_2 (see text). The neutral radical is represented by △, and ▲ is obtained from (○ − △).

could not be observed at pH ≤ 2.0. Another somewhat longer lived spectrum is also observed (formed during the 15 nsec laser pulse) with maxima at ~445 nm and <235 nm and weaker bands at ~560 and ~340 nm. At 100 nsec after the pulse, a large decrease in the absorbance of the 445 nm band is found (Figure 5a). The difference spectrum with $\lambda_{max} \sim 440$ nm is referred to as transient T_1.

The spectrum obtained at 100 nsec after the pulse in O_2-free solutions is shown in Figure 5b. The disappearance of this 450 nm band (referred to as T_2) parallels the decrease in absorbance at $\lambda < 260$ nm. As with indole, the band at 560 nm, assigned to the cation radical, decay with $k \sim 10^6$ sec^{-1} in neutral solution.

The T_2 spectrum is very similar to the T-T spectrum of indole (see more below) and is assigned to the T-T absorption of tryptophan at pH 7.0 (zwitterion form). It decays with $k = 7.0 \pm 0.7 \times 10^4$ sec^{-1} at pH 7.5 (see Table I) and is quenched by oxygen with $k_q = 5.0 \pm 1.0 \times 10^9$ M^{-1} sec^{-1} (Table II). It can also transfer its energy to anthracene. This experiment was carried out in ethyl alcohol, and the *formation* kinetics of triplet anthracene was monitored at 428 nm. The rate constant for T-T energy transfer, $k_4 = 4.0 \pm 0.4 \times 10^9$ M^{-1} sec^{-1}, was determined.

$$^3\text{Trp} + \text{anthracene} \longrightarrow \text{Trp} + {}^3\text{anthracene} \quad (4)$$

The ^{3}Trp is also quenched by disulfides (RSSR) and the rate constant for lipoate ion, a cyclic disulfide, is $k_q = 3.6 \pm 0.4 \times 10^9$ M^{-1} sec^{-1} (Table II). The quenching mechanism was found to occur by transfer of an electron with the formation of the $RSSR\cdot^-$ radical anion.

$$^3\text{Trp} + \text{RSSR} \longrightarrow \text{Trp}^{\cdot+} + \text{RSSR}^{\cdot-} \quad (5)$$

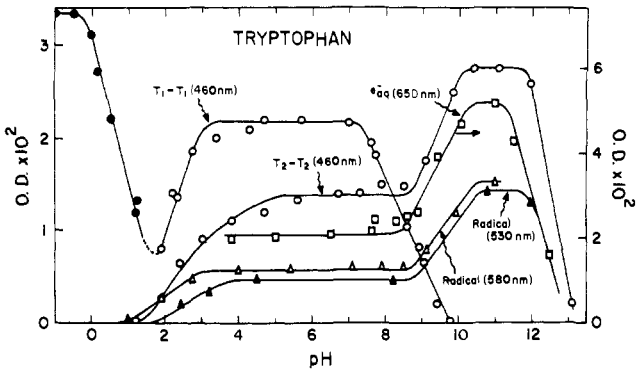


Figure 6. Titration curves of the transient species produced in the laser photolysis of 1.5×10^{-4} M tryptophan (25°) in O_2-free water. OD read at 0 nsec (○ and ●), at 100 nsec for T_2 (○), at 20 nsec for □ and △, and at 1 μsec (in O_2-saturated solution) for ▲ after the laser pulse.

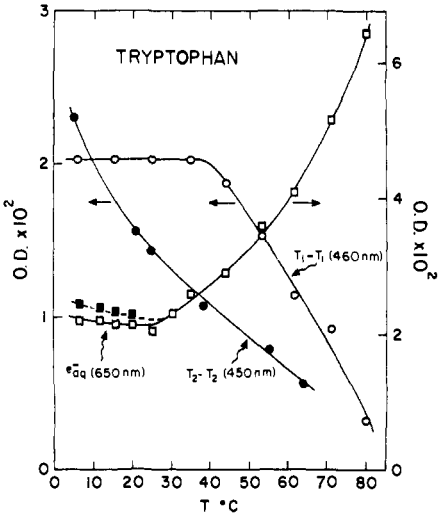


Figure 7. Dependence upon temperature of the yields of transient species produced from the laser photolysis of tryptophan (1.5×10^{-4} M) at pH 7.6.

The characteristic[43] spectrum of RSSR·⁻ with $\lambda_{max} \sim 420$ nm was observed. As was found[33,44] for the quenching of the triplet states of tyrosine and tyrosine peptides by RSSR compounds, the efficiency of the charge transfer reaction 5 is ≪100%. However, the quenching of the triplet states of these aromatic amino acids by vicinal disulfide linkages in proteins may be an important process, possibly resulting in the rupture of the -S-S- bridges, since the cyclic RSSR·⁻ radical is known[43] to break apart.

$$RSSR\cdot^- \rightleftharpoons RS\cdot + RS^- \quad (6)$$

In the presence of RS^- ions an equilibrium can be reached.

The population of the triplet state of tryptophan (T_2 spectrum) by energy transfer from ^{3}Tyr was observed[33] in

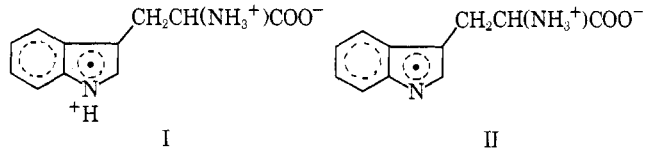
$$^3Tyr + Trp \longrightarrow Tyr + {}^3Trp \quad (7)$$

water at pH 7.3. This was demonstrated by monitoring the formation kinetics of ^{3}Trp at 450 nm. At this wavelength the absorption of ^{3}Tyr is relatively weak. A value of $k_7 = 6.0 \pm 1.5 \times 10^9$ M^{-1} sec^{-1} was derived, based on three concentrations of tryptophan.

The T_1 spectrum mentioned above will be discussed separately below. The tentative conclusion reached is that it is another excited state, possibly a triplet state.

Following the decay of T_2 in neutral solution, a longer lived absorption with maxima at 530, 330, and <230 nm is observed. In O_2-saturated solutions, an identical absorption is observed after the decay of T_2 and the cation radical (Figure 5b). This absorption is attributed to the neutral radical of tryptophan.

The tryptophan cation radical (I) produced from the photoionization reaction has maxima at ~560 and ~330 nm, Figure 5, in agreement with an earlier[30] assignment. As was found for indole, its lifetime in neutral solutions is relatively short, $\tau \sim 1 \times 10^{-6}$ sec, and in alkaline solutions it is much shorter lived due, presumably, to loss of a proton to form the neutral radical (II). In acidic solutions at pH 3-4,



I II

it is relatively long lived, $\tau > 100$ μsec. As in the case of indole, there was no experimental evidence for the formation of radical II from the decay of radical I. Radical II is suggested to be a "nitrogen radical" with the odd electron largely localized on nitrogen, for the same reasons given above for indole. Furthermore, on excitation of 1-methyltryptophan the cation radical was observed[30] but no absorption band from the decay of this radical could be observed. With 1-Me-Trp the neutral radical cannot be a nitrogen-centered radical, and the carbon radical formed is expected to absorb at $\lambda < 350$ nm and have a relatively low extinction coefficient.

Dependence upon pH. Figure 6 shows the dependence upon pH of the triplets (T_1 and T_2 transients), e_{aq}^-, cation radical, and neutral radical produced from the optical excitation of tryptophan in water at 25°. The quantum yield of e_{aq}^- and, therefore, those of the cation and neutral radicals show a plateau in the pH range ~3.0-8.5. At pH >8.5, an increase in ϕ is observed reaching another plateau at pH ~10-11.5, with an increase in ϕ of a factor of ~2-2.5. The apparent $pK_a \sim 9.3$ appears to correspond to the ionization of the NH_3^+ group in Trp. At pH <3.0 the decrease in ϕ appears to correspond to the pK_a for protonation of the COO^- group in Trp. These titration curves are the same as that observed for ϕ_F. At pH 2.3, ^{3}Trp decays with $k = 5.7 \pm 0.5 \times 10^5$ sec^{-1} (Table I).

The T_2 transient of ^{3}Trp also follows the same changes with pH. It is, however, *not* the precursor for the electron ejection from tryptophan since the e_{aq}^- (and the radicals) is produced within the laser pulse and not from the decay of ^{3}Trp. Furthermore, as will be shown below, the photoionization of Trp does not occur via a biphotonic process from the triplet state, as was found for tyrosine[33,45,46] and phenylalanine.[36,47] At pH 11.0, ^{3}Trp decays with $k = 5.0 \pm 1.0 \times 10^4$ sec^{-1} (Table I). At pH >11.5 a sharp decrease in ϕ is found.

The T_1 species, Figure 6, shows a different titration curve. Its yield decreases to zero in slightly alkaline solutions with an apparent $pK_a \sim 8.5$. In acid solutions at pH <3.0, its yield decreases also. At pH below ~1.5 a change in the titration curve is observed. A different shorter lived transient with a $pK_a \sim 1.0$ is formed and its spectrum is shown in Figure 3. The spectral characteristics and its formation are very similar to those reported above for indole, indicating that the side chain in Trp is not involved. Tryptophan has a ground state, $pK_a = -6.23$, protonation occurring on the 3 position, and $\phi_F = 0.072$[22] for $TrpH^+$. It is suggested that the spectrum shown in Figure 3 is that of $^3TrpH^+$. At pH 0.2, the transient decays with $k = 3.0 \pm 0.3 \times 10^7$ sec^{-1} (Table I). No other transient absorptions were observed in 1-3 M H_2SO_4 solutions or from the decay of $^3TrpH^+$, indicating the absence of electron ejection.

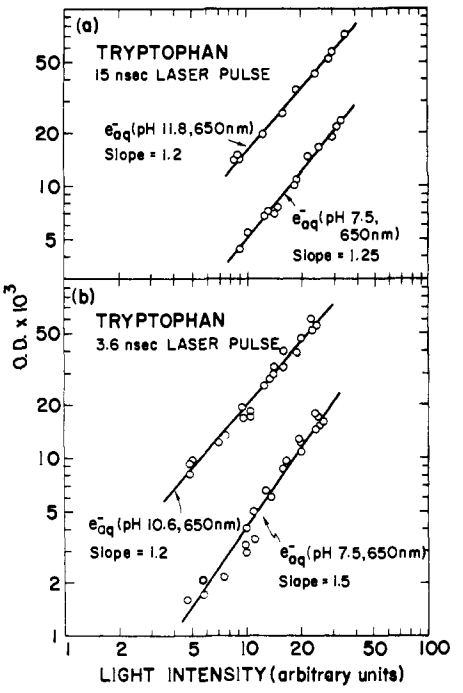


Figure 8. Dependence upon the 265 nm light intensity of the yield of hydrated electrons produced on laser photolysis of tryptophan (1.5×10^{-4} *M*, 25°) using a 15 nsec and a 3.6 nsec laser pulse. Note the difference in the slopes with change in pH and in pulse duration.

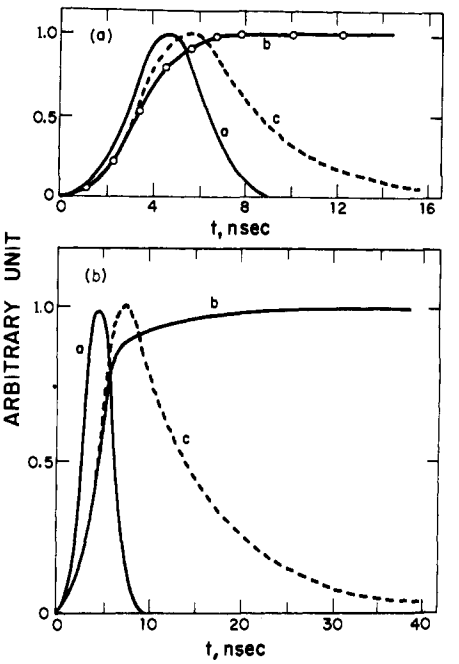


Figure 9. Laser photolysis of tryptophan (1.5×10^{-4} *M*) at (a) pH 6.8 and (b) pH 10.3. In each case, (a) represents the flash profile, (b) shows the formation of e_{aq}^- at 650 nm as a function of time, and (c) shows the decay of the fluorescence of Trp monitored at 353 nm (for pH 6.8) and 360 nm (for pH 10.3). In addition, in (a) the open circles represent the integrated laser pulse. Curves were normalized for ease of comparison.

Dependence upon Temperature. The effect of temperature on the fluorescence intensity of Trp in water is considerable,[19,48] with ϕ_F decreasing with increase in T over the range 5-95°, while the emission spectrum remains unchanged.

Figure 7 shows the results obtained on optical excitation of Trp (1.5×10^{-4} *M*) in water at pH 7.6. The yield of the T_2 transient of ^{3}Trp decreases monotonically with an increase in temperature over the range 5-65°. This is qualitatively similar to the decrease in ϕ_F. The T_1 species, however, shows no temperature dependence from 5 to 35° but its yield decreases above 35°.

The yield of e_{aq}^- shows interesting changes with temperature. Below 25° there is very little change in the yield of e_{aq}^- observed 20 nsec after the laser flash. A slightly larger yield of e_{aq}^- is, however, observed during the laser flash. This additional absorption at 650 nm decays with $\tau < 10$ nsec due, possibly, to recombination with the cation radical. Above 25° the fast decay of e_{aq}^- is not observed, but a sudden large increase in $\phi_{e_{aq}^-}$ occurs. As mentioned above for indole, the ϵ_{650} of e_{aq}^- decreases with an increase in temperature.

Dependence upon Light Intensity. The photoionization of tyrosine and phenol compounds,[33,45,46] and of phenylalanine and related compounds,[36,47] in water was shown to occur from the triplet state of these molecules via a biphotonic process. A dependence upon the (light intensity)^2 for excitation of these compounds was established.

A logarithmic plot of $OD_{e_{aq}^-}$ as a function of the laser energy (15 nsec pulse) for excitation of Trp at pH 7.5 and 11.8 at 25° gave a slope of 1.2, Figure 8a. This is less than for a biphotonic process (slope = 2) and more than for a monophotonic process (slope = 1). When using a narrower 3.6 nsec laser pulse, a slope = 1.5 at pH 7.5 and slope = 1.2 at pH 10.6 were found, Figure 8b. Furthermore, in alkaline solutions using the 3.6 nsec pulse, ~10% of the total yield of e_{aq}^- was formed *after* the end of the laser pulse. Similar observations were more difficult in neutral solution due to the much shorter lifetime of ^{1}Trp*, but it is clear that e_{aq}^- is produced mainly within the laser flash.

Figure 9 shows the results obtained on laser photolysis of Trp at pH 6.8 and 10.3 using a pulse of 3.6 nsec duration. It is apparent from these curves that: (a) at pH 10.3, τ_F is 9.0 ± 0.4 nsec, as compared to ~3.0 nsec at pH 6.8; (b) the formation of e_{aq}^- (i.e., the photoionization of Trp) at pH 6.8 follows the integrated laser pulse and is not produced from the decay of the lowest excited singlet state; and (c) at pH 10.3, only ~10% of the total yield of e_{aq}^- appears to be formed from the decay of the lowest excited singlet state.

The following additional remarks can be made: (a) the singlet state is the main precursor leading to the photoionization of tryptophan in water; (b) e_{aq}^- is formed by a predominantly monophotonic process; (c) since ~10% or less of the total $\phi_{e_{aq}^-}$ is formed *after* the 3.6 nsec laser pulse, only ~10% of e_{aq}^- is produced from the lowest excited singlet state of Trp; (d) an upper excited singlet excited state is therefore suggested for the remaining ~90% which is formed within the 3.6 nsec laser pulse; (e) the very strong temperature dependence of $\phi_{e_{aq}^-}$ above 25° (Figure 7) could be due to photoionization from a vibrationally excited singlet state (the ionization potential of indole in the gas phase is 7.86 eV;[48] this could be lowered by up to ~2 eV due to solvation effects and/or to different solvent states); (f) at pH 7.5, the increase in the slope from 1.25 to 1.5 (Figure 8) can be due to a biphotonic process from the singlet excited state (the triplet state probably does not contribute very much in this biphotonic mechanism, under these experimental conditions, since the increase in the slope cannot be explained based on the lifetime of the triplet state); (g) using the 3.6 nsec pulse, the slope = 1.2 at pH 10.6 as compared to 1.5 at pH 7.5, is probably related to the ionization of the $-NH_3^+$ group (no other explanation is presently available).

Tryptophan Derivatives and Tryptophanyl Peptides. On optical excitation of tryptamine, two species corresponding to T_1 and T_2 were observed, as found for Trp. The T_1 triplet

decays with $k = 3.0 \pm 1.0 \times 10^7$ sec^{-1} and T_2 with $k = 7.1 \pm 0.7 \times 10^4$ sec^{-1} at pH 7.5 (see Table I). The T_2 triplet is quenched by O_2 with $k_q = 5.7 \pm 1.0 \times 10^9$ M^{-1} sec^{-1} (Table II).

The short lived T_1 transient was not observed with *N*-methyltryptophan. The triplet observed (corresponding to T_2 for Trp) decays with $k = 7.5 \pm 0.8 \times 10^4$ sec^{-1} at pH 7.5 and is quenched by lipoate ion with $k_q = 3.1 \pm 0.2 \times 10^9$ M^{-1} sec^{-1} (Tables I and II). *N*-Acetyltryptophan also showed only one triplet absorption at ~440 nm with $k = 6.1 \pm 0.1 \times 10^4$ sec^{-1}.

The peptide tryptophanyl glycine showed two transient species. T_1 decays with $k = 5.0 \pm 2.5 \times 10^7$ sec^{-1} and T_2 decays with $k = 6.2 \pm 0.6 \times 10^4$ sec^{-1}. The T_2 triplet is quenched by oxygen with $k_q = 4.0 \pm 1.0 \times 10^9$ M^{-1} sec^{-1}.

The dipeptide glycyl tryptophanyl glycine showed only a T_2 absorption which decayed with $k = 8.5 \pm 2.0 \times 10^4$ sec^{-1}, and was quenched by oxygen with $k_q = 4.5 \pm 1.0 \times 10^9$ M^{-1} sec^{-1}.

Nature of the T_1 Transient. The following remarks and conclusions can be made. (a) It is suggested that T_1 is an excited state, possibly a triplet state since its absorption spectrum is so similar to transient T_2 which has been shown to be a triplet state of tryptophan (it is quenched by O_2 and by RSSR, can be populated by energy transfer from ^{3}Tyr and can populate ^{3}anthracene by energy transfer to anthracene). Its lifetime of ~44 nsec (independent of pH from 2 to 9.5) is too long for the singlet excited state. (b) The T_1 transient is observed only when an amino group is present (it is not observed in indole and indole-3-propionic acid) in its protonated NH_3^+ form. It is observed on excitation of tryptamine at pH 7.5 and of tryptophanylglycine at pH 5.0, but not on excitation of Trp at pH ≥9.5 (see Figure 6), *N*-methyltryptophan, *N*-acetyltryptophan, and Gly-Trp-Gly. (c) T_1 is not observed on excitation of Trp in methyl alcohol solutions, since presumably under these conditions the pK_a* and solvent states are different. (d) Its singlet excited state precursor is presumably different from that which gives rise to the T_2 triplet state, since its temperature dependence (Figure 7) is quite different. (e) From (d) above it follows that T_1 is not the precursor of T_2. (f) No transient optical absorption could be seen to arise from the decay of T_1 (or from T_2). (g) The absence of a T_1 species in indole, indole-3-propionic acid, *N*-Me-Trp, *N*-Ac-Trp, Gly-Trp-Gly, and Trp at pH ≥9.5 may be due to either the need of a NH_3^+ for its formation or, in the absence of the NH_3^+ group, it is too short lived ($\tau < 5$ nsec) for observation under the experimental conditions used. It is possible that the NH_3^+ group creates a charge transfer interaction in the electronic transition giving rise to the population of the T_1 transient. (h) Dimerization of tryptophan is not known; therefore, we rule out any formation of excimers. (i) The existence in indole and Trp of two fluorescent states ^1L_A and ^1L_B is well known,[3-10] but no fluorescence with $\tau \sim 44$ nsec has been observed. Therefore, if T_1 is an excited singlet state it must decay via a radiationless transition. Futhermore, T_1 was not observed in indole.

Conclusions

The triplet absorption spectra and lifetimes of indole, tryptophan, and related derivatives and peptides in water have been determined for the first time in fluid solutions. For tryptophan and a few tryptophanyl derivatives (see above), but not for indole, two transients have been observed: T_1 is short lived with $\tau_{T_1} \sim 20$-45 nsec, and T_2 is longer lived with $\tau_{T_2} \sim 15$ μsec. Transient T_1 is not the precursor of T_2.

For indole $\tau_T = 11.6$ μsec, while substitution in the 3 position lengthens the lifetime to $\tau_T = 15.1$ μsec for indole-3-propionic acid and $\tau_{T_2} = 14.3$ μsec for tryptophan (zwitterion form). For the dipeptide Gly-Trp-Gly the triplet is shorter lived with $\tau_T = 11.8$ μsec. Quenching of the triplet states by disulfide compounds and by oxygen is very efficient with $k_q \sim 4$-6×10^9 M^{-1} sec^{-1}. For disulfides, evidence has been obtained for the nature of the quenching mechanism: the electron transfer reaction 5 was found to occur, but with a low efficiency.

$$^3\text{Trp} + \text{RSSR} \longrightarrow \text{Trp}^{\bullet+} + \text{RSSR}^{\bullet-} \quad (5)$$

The photoionization process for indole, tryptophan, and related derivatives and peptides is a major reaction leading to a chemical change in the chromophore. This is comparable to the photoionization reactions occurring with the other aromatic amino acids, tyrosine,[33,45,46] and phenylalanine.[36,47]

The precursor leading to electron ejection in the indoles is indicated to be an upper excited singlet state and/or a vibrationally excited lowest singlet excited state. The photoionization occurs primarily via a monophotonic process with only a small contribution from a biphotonic process, under the experimental conditions used (3.6 nsec pulse duration and excitation at 265 nm). It should be stated that under other optical excitation conditions (e.g., nonmonochromatic light) a biphotonic process from the triplet state of indoles could occur in fluid solutions. It was not observed in this work because at 265 nm the ground state of indoles absorbs strongly and the main T-T absorption of indoles is at ~440-450 nm. Biphotonic processes from ^{3}Trp have been observed in low-temperature organic glasses.[49,50] Similarly, a biphotonic process from the lowest excited singlet state may become a more important reaction on excitation by nonmonochromatic light.

Other photoinduced changes (e.g., decarboxylation) may have occurred which could not be observed by this technique or under the experimental conditions used.

Table III shows a compilation of the relative quantum yields for the photoionization of indoles. The following interesting points can be made: (a) indole and indole-3-propionic acid give the highest $\phi_{e_{aq}^-}$ yields, and are independent of pH up to pH 11.0; (b) methylation of the -NH group in

Table III. Relative Yields of the Photoionization in Water at 25° of Indole, Tryptophan, and Related Peptides, Optically Excited at 265 nm

Compd	pH	$\phi_{e_{aq}^-}$[a]	Ratio[b]
Indole	6.0	0.26	3.3
	11.0	0.27	3.4
Indole-3-propionic acid	6.5	0.26	3.3
	10.5	0.26	3.3
1-Methylindole	5.6	0.17	2.1
	10.7	0.17	2.1
Tryptophan	6.0	0.08	1.0
	11.0	0.21	2.6
Tryptamine	5.2	0.12	1.5
	10.5	0.19	2.4
N-Methyltryptophan	6.2	0.09	1.1
	10.9	0.09	1.1
N-Acetyltryptophan	7.0	0.19	2.4
	10.8	0.19	2.4
Tryptophanylglycine	5.2	0.05	0.6_2
Glycyltryptophan	5.0	0.03	0.3_8
	10.9	0.07	0.8_8
Glycyltryptophanylglycine	5.2	0.04	0.5
	10.9	0.06	0.7_5

[a] Determined from solutions whose absorbance at 265 nm was 0.90 ± 0.05, and derived by monitoring e_{aq}^- at 675 nm at "zero" time after the laser pulse. The relative yields are considered to be of greater significance. [b] Ratio based on e_{aq}^- yield from tryptophan at pH 6.0.

Table IV. Quantum Yields for the Photoionization of Aromatic Amino Acids and Peptides in Water at 25° Optically Excited at 265 nm

Peptide	pH	$\phi_{e_{aq}^-}$[a]	Ref
Tyrosine	7.5	0.095	33
Tyrosylglycine	6.0	0.051	33
Glycyltyrosylglycine	6.0	0.035	33
Phenylalanine	7.5	0.034	36
Glycylphenylalanylglycine	5.0	0.038	36
Tryptophan	6.0	0.08	This work
Tryptophanylglycine	5.2	0.05	This work
Glycyltryptophan	5.0	0.03	This work
Glycyltryptophanylglycine	5.2	0.04	This work

[a] The relative yields are considered to be of greater significance.

indole reduces $\phi_{e_{aq}^-}$; (c) for Trp at pH 6.0, the $\phi_{e_{aq}^-} = 0.08$ and increases to 0.15 on deprotonation of the NH_3^+ group; (d) *N*-Me-Trp and *N*-Ac-Trp show no pH dependence of the yield of e_{aq}^-, as expected; and (e) the yields of e_{aq}^- from the peptides Gly-Trp and Gly-Trp-Gly are much lower than that of Trp, and show a pH dependence.

Table IV summarizes the $\phi_{e_{aq}^-}$ produced from the photoionization of aromatic amino acids and the corresponding peptides. These yields were all determined under similar experimental conditions. The relative yields are considered to be of greater significance.

The photoionization of tryptophan when present in proteins could have various consequences due to the interaction of e_{aq}^- (or the nonsolvated electron e^-) with (a) vicinal -S-S- bridges, leading to the formation[43] of $RSSR\cdot^-$ and S-S bond rupture

$$e_{aq}^- + RSSR \longrightarrow RSSR\cdot^- \quad (8)$$

$$RSSR\cdot^- \rightleftharpoons RS\cdot + RS^- \quad (6)$$

(in addition to reaction 5); (b) the peptide linkage,[51] leading to the formation of ketyl radicals. These radicals have[52] very low kinetic potentials (i.e., are strong reducing agents)

$$e_{aq}^- + -CONH- \longrightarrow -\dot{C}(OH)NH- + OH^- \quad (9)$$

and could transfer an electron readily along the peptide chain or to other constituents in the protein, e.g., methionine,[53] tyrosine,[46] phenylalanine,[54] and histidine.[55]

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