Electron-Transfer Reactions of Tryptophan and Tyrosine Derivatives

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Oxidation of tryptophan, tyrosine, and their derivatives by oxidizing radicals was studied by pulse radiolysis in aqueous solutions at 20 °C. Rate constants for the oxidation of tryptophan derivatives with $\cdot N_3$ and $Br_2^{-}\cdot$ radicals vary from 8×10^8 to 4.8×10^9 M^{-1} s^{-1} and oxidation goes to completion; no pH dependence was observed. Oxidation rate constants for tyrosine derivatives increase upon deprotonation of the phenolic residue at higher pH. Redox potentials for the indolyl and phenoxyl radicals were derived from the measured equilibrium constants by using *p*-methoxyphenol ($E_{7.5} = 0.6$ and $E_{13} = 0.4$ V), bisulfite ($E_3 = 0.84$ V), and guanosine ($E_7 = 0.91$ V) redox couples as reference systems. The redox potential of the tryptophyl radical was measured by pulse radiolysis and laser photolysis and found, by both techniques, to be $E = 0.64$ V at pH 7. Redox potentials of tryptophan derivatives were found to be dependent on the nature of the side chain possibly due to interaction of the side chain with the nitrogen atom in the pyrrole ring. Redox potentials of tyrosine derivatives were found to be independent of the nature of the side chain and higher than the redox potentials of tryptophan derivatives. $E = 0.85$ V and $E_{13} = 0.65$ V were measured for the tyrosine/phenoxyl radical redox couple at pH 7 and 13, respectively. Electron transfer from tyrosine to tryptophyl radical was found to be slow in neutral media, $k = 5 \times 10^5$–1.3×10^6 M^{-1} s^{-1}, and is suggested to proceed via multiple steps, one of which is proton transfer from tyrosine to tryptophyl radical followed by electron transfer.

Introduction

Radiation damage to proteins in general and enzymes in particular has many biological and technological consequences.[1] The initial damage to a particular amino acid may, under certain conditions, migrate to another one. Possible intramolecular electron-transfer processes associated with migration of damage involving tryptophan and tyrosine in model peptides and proteins have been extensively studied.[2-5] Redox potential values for the redox couples Trp/Trp·, H^+ and Tyr/Phe–O· were evaluated[5] as 0.94 and >0.8 V at pH 7.0, respectively. However, the value of the redox potential for tryptophan was not in agreement with the value of ~0.6 V obtained under somewhat different conditions.[6]

Tyrosinephenoxyl radicals have also been implicated in radiation-induced cross-links between DNA and proteins,[7] and the question arises as to whether initial damage to tryptophan, which has not been shown to cross-link readily, may induce cross-linking through migration of damage to tyrosine.

Radiation and oxidative damage to DNA repair enzymes is still an area where relevant information is lacking. How efficient are various radicals in inactivation of these enzymes and whether the damaged sites can be repaired by electron or H-atom transfer from various physiological donors are questions of major importance in the preservation of the full capacity of the DNA repair enzyme system.

Very recently, the Food and Drug Administration has approved radiation sterilization of enzymes used in food processing. Here again, questions associated with radiation-induced damage to enzymes and possible reduction of the damage by repair are important in radiation technology.

In view of the widespread interest in migration of damage and damage repair in proteins in general, measurements of the redox potentials of tryptophan and tyrosine and their derivatives were undertaken and the effects of structure and pH are presented.

Materials and Methods

Chemicals were of the highest purity available and were used without further purification. Tryptophan and tyrosine were obtained from Vega,[24] *N*-acetyl-L-tryptophan and sodium azide were from Aldrich, DL-tryptophanamide, *p*-methoxyphenol, D-tryptophyl methyl ester·HCl, DL-tyrosine methyl ester·HCl, *N*-acetyl-L-tyrosinamide, L-tryptophyl-L-alanine, L-tyrosil-L-alanine, L-alanyl-L-tryptophan, guanosine, and L-alanyl-L-tyrosine were from Sigma, sodium bisulfite was from Fisher, and tryptamine was from Eastman Kodak. Water was purified by a Millipore Milli-Q system and solutions were freshly prepared before each experiment. The pH was adjusted where necessary with sodium hydroxide and perchloric acid or was maintained with phosphate and borate buffers.

The pulse radiolysis experiments were conducted on the Febetron 705 pulse radiolysis setup (described in ref 8), which allowed single-pulse transient absorption spectra measurements and simultaneous absorbance vs. time readings at a fixed wavelength with the lowest time resolution of 1 μs. A Suprasil quartz cell with 2-cm optical path length was used in all experiments. Doses were in the range of 2 to 50 Gy, as determined by thiocyanate dosimetry.

Reactivities of the bromide radicals with solutes were determined from the effect of various concentrations of solutes on the decay of absorbance at 355 nm, which was previously ascribed to $Br_2^{-}\cdot$.[9] Reactivities of the azide radicals with solutes were determined from the pseudo-first-order buildup of absorbances at 525 nm for indole derivatives and 405 nm for tyrosine derivatives. In a typical experiment, an N_2O-saturated 0.1 M aqueous solution of either KBr or NaN_3 and a solute at concentrations of up to 10 mM was irradiated with a pulse of 2 Gy. The bromide or azide radicals are formed under these conditions within a fraction of a microsecond and decay in the absence of any other solutes on a millisecond time scale. However, when reactive solutes were added in appropriate concentrations, the decay was changed to a microsecond time scale. The concentration of solutes was varied within an order of magnitude and from the pseudo-first-order rate constants thus obtained, second-order rate constants were derived.

Results and Discussion

Oxidation of tryptophan, tyrosine, and their derivatives was carried out using strongly oxidizing radicals of bromide, $Br_2^{-}\cdot$,

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TABLE I: Reactivities of Oxidizing Radicals with Tryptophan and Tyrosine Derivatives

	$k(\cdot N_3+S)^a$		$k(Br_2^{-}\cdot+S)^a$	
solute, S	pH 7.5	pH 13	pH 7	pH 13
tryptophan	4.1×10^{9b}	4.4×10^{9b}	7.7×10^{8c}	7.7×10^{8c}
tryptamine	4.3×10^{9}	4.3×10^{9}	1.6×10^{9}	1.6×10^{9}
tryptophan methyl ester	4.2×10^{9}	4.2×10^{9}	8.3×10^{8}	8.3×10^{8}
tryptophanamide	4.8×10^{9}	4.8×10^{9}	1.1×10^{9}	1.1×10^{9}
N-acetyltryptophan	4.4×10^{9}	4.4×10^{9b}	8.3×10^{8}	8.3×10^{8b}
tryptophyl-alanine	4.4×10^{9}	4.4×10^{9}	*e*	*e*
tyrosine	1.0×10^{8c}	3.6×10^{9c}	2×10^{8d}	5×10^{8d}
N-acetyltyrosinamide	1.26×10^{8}	*e*	*e*	7.2×10^{8b}
tyrosine methyl ester	1.5×10^{8}	4×10^{9}	1.5×10^{7}	1.5×10^{9}

[a] Rate constants in units M^{-1} s^{-1}, estimated to be accurate to ±10%. [b] Determined at pH 12 in order to avoid alkaline hydrolysis of the side chain. [c] From ref 16. [d] The same as in ref 15. [e] Not measured.

and azide, $\cdot N_3$, whose one-electron redox potentials are 1.7[10] and 1.90 V,[11] respectively. Since the sites of attack of the oxidizing radicals are indole in tryptophan and its derivatives and phenol in tyrosine and its derivatives, indole and phenol are high lighted in the abbreviated formulas used throughout this study. The mechanism of one-electron oxidation of the tryptophan derivatives can be envisaged as follows:

$$\cdot X + R\text{–}IndH \rightarrow R\text{–}IndH^{+}\cdot + X^{-} \quad (1)$$

$$R\text{–}IndH^{+}\cdot \rightleftharpoons R\text{–}Ind\cdot + H^{+} \quad (2)$$

$$pK_a = 4.3 \quad \text{(ref 12)}$$

whereas the corresponding mechanism of the oxidation of the tyrosine derivatives is

$$\cdot X + R\text{–}PhOH \rightarrow R\text{–}PhOH^{+}\cdot + X^{-} \quad (3)$$

$$R\text{–}PhOH^{+}\cdot \rightleftharpoons R\text{–}PhO\cdot + H^{+} \quad (4)$$

$$pK_a < 0 \quad \text{(ref 13)}$$

$$\cdot X + R\text{–}PhO^{-} \rightarrow R\text{–}PhO\cdot + X^{-} \quad (5)$$

$$R\text{–}PhOH \rightleftharpoons R\text{–}PhO^{-} + H^{+} \quad (6)$$

$$pK_a = 10.47 \quad \text{(ref 14)}$$

$\cdot X$ and X^{-} are used to abbreviate the oxidized and reduced forms of oxidizing radicals. The rate constants for reactions 1, 3, and 5 were determined by using the procedure outlined in the Materials and Methods section, and the results are summarized in Table I.

The rates of oxidation of the tryptophan derivatives by oxidizing radicals were in the range of 7×10^{8}–4.8×10^{9} M^{-1} s^{-1}. No significant pH dependence of the rate constants was observed in the pH range 3–14. Reactivities of the azide radicals with the tryptophan derivatives were similar but higher than corresponding values for the $Br_2^{-}\cdot$ reactions.

Reactivities of oxidizing radicals with the tyrosine derivatives were lower than those of the tryptophan derivatives, especially in neutral media. The rates of one-electron oxidation of deprotonated tyrosine derivatives, reaction 5, were found to be higher by one or even two orders of magnitude than those of R–PhOH.

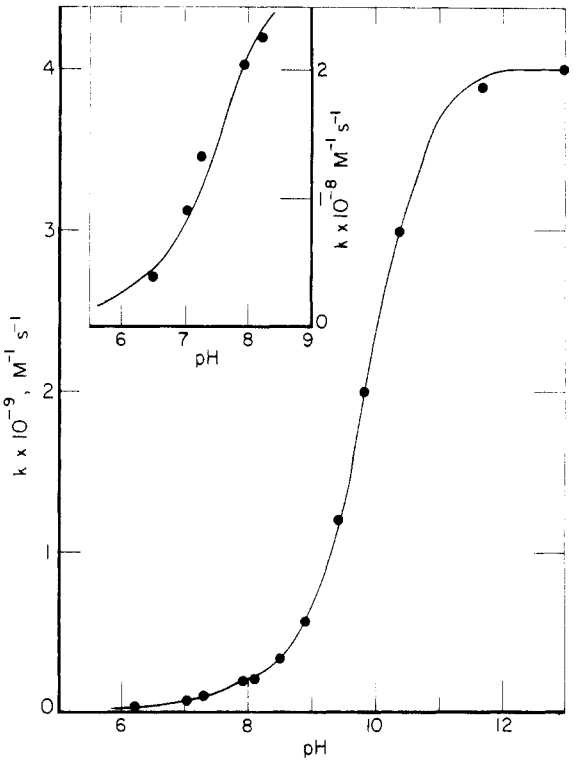

Figure 1. pH dependence of the rate of oxidation of tyrosine methyl ester by the azide radicals. The solid curve is drawn through experimental points obtained by assuming $pK_{a1} = 7.3$ (amino group in the side chain) and $pK_{a2} = 9.9$ (phenyl group), and $k_1 = 2 \times 10^{7}$, $k_2 = 2 \times 10^{8}$, and $k_3 = 4 \times 10^{9}$ M^{-1} s^{-1} for oxidation of respectively positively charged, neutral, and negatively charged tyrosine methyl ester. Inset shows the enlarged portion of the above pH dependence so that the first inflection point may be observed.

This may be rationalized on the basis of the higher electron-donating ability of the phenoxide ion. The dependence of oxidation rates upon pH actually follows the titration curve of the phenoxide ion[15] which supports the above premise. However, in the oxidation of tyrosine methyl ester by oxidizing radicals, the pH dependence of rate constants revealed two pK_a values, as shown in Figure 1. The first $pK_a = 7.3$ is that of amino group in the side chain, whereas the second $pK_a = 9.9$ corresponds to the formation of the phenoxide ion.

In all instances, the reactions of oxidizing radicals with the tryptophan and tyrosine derivatives were found to proceed to completion. This was verified by changes in the absorption spectra for $Br_2^{-}\cdot$ mediated reactions and by comparison of the spectra of transients produced either by oxidizing radicals or by flash photolysis of aqueous solutions of the derivatives studied. The spectra of transients obtained by one-electron oxidation of tryptophan were similar to those previously reported for indolyl radicals,[12,15,16] whereas the spectra of the oxidized tyrosine derivatives were characteristic of phenoxyl radicals.[15,16] The spectral and acid–base properties of the indolyl and phenoxyl radicals were found to be invariant with changes in the side chain of the parent compounds.

One-electron oxidations of the tryptophan and tyrosine derivatives result in fairly strong oxidizing agents, the indolyl and phenoxyl radicals. In order to obtain the redox properties of those radicals, we investigated electron-transfer (ET) reactions involving the indolyl or phenoxyl radicals with various electron donors. For convenience, the reactions studied were divided into two groups on the basis of the mechanism of electron transfer. The first group includes ET reactions with predominantly equilibrium kinetics, which were used for the determination of one-electron redox potentials of the derivatives studied. In a separate section, we

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TABLE II: One-Electron Redox Reactions of Tryptophan and Tyrosine Derivatives, Measured at 20 °C

$$R\cdot + A^- \underset{k_r}{\overset{k_f}{\rightleftharpoons}} R^- + A\cdot$$

R· from	A⁻	pH	k_f,[a] M^{-1} s^{-1}	k_r,[a] M^{-1} s^{-1}	K_{kin}[b]	K_{abs}[b]	E,[c] V
tryptophan	HSO_3^-	3.0	4.2×10^6	8×10^4	52	*f*	0.1
	p-methoxyphenol	7.5	5.5×10^6	1×10^6	5.5	6	0.04
	guanosine	7.0	*f*	3.4×10^8	*d*	*d*	>−0.2
	p-methoxyphenol	13.0	3.6×10^7	1×10^5	360	570	0.16
tryptophanamide	HSO_3^-	3.0	2.2×10^7	4×10^5	55	*f*	0.1
	p-methoxyphenol	7.5	1.5×10^7	6.9×10^5	22	24	0.08
	p-methoxyphenol	13.0	1.5×10^8	*f*	*d*	*d*	>0.2
	N-acetyltyrosinamide	12.0	3×10^6	9×10^6	0.33	0.22	−0.03
N-acetyltryptophan	HSO_3^-	3.0	$<5 \times 10^5$	$<5 \times 10^5$	*e*	*e*	<+0.03
	p-methoxyphenol	6.5	3.1×10^7	8×10^5	39	40	0.09
tryptamine	HSO_3^-	3.0	7.8×10^6	5.1×10^4	153	*f*	0.13
	p-methoxyphenol	7.5	4.8×10^6	9×10^5	5.4	6	0.04
	p-methoxyphenol	13.0	4.2×10^7	1×10^5	420	550	0.16
tryptophan methyl ester	*p*-methoxyphenol	7.5	2.1×10^7	9×10^5	23	24	0.08
tryptophyl-alanine	*p*-methoxyphenol	7.5	1.8×10^7	1.1×10^6	16	20	0.07
tyrosine	tryptophan	13.0	2.4×10^6	1×10^5	24	22	0.08
N-acetyltyrosinamide	tryptophan	12.0	9×10^6	2×10^5	45	20	0.09
tyrosine methyl ester	tryptophan	13.0	5×10^6	1×10^5	50	52	0.1
	guanosine	7.0	5×10^6	8.9×10^6	0.56	0.1	−0.04
alanyl-tyrosine	guanosine	7.0	1×10^6	1×10^7	0.1	0.1	−0.06

[a] Rate constants determined from the kinetics, estimated to be accurate to ±10% for reactions proceeding in the favorable direction, ±20% for the others. [b] Equilibrium constants derived from the kinetics and absorbances of radicals at equilibrium. [c] The difference in one-electron redox potentials calculated from the average of equilibrium constants (for the formula see text). Estimated to be accurate to ±0.02 V. [d] The reaction was found to proceed to completion, presumably as a consequence of high redox potential difference. [e] The reaction in either direction was too slow to be measured, hence only an estimate of the redox potential difference could be made. [f] Not measured.

discuss the ET reactions between indolyl radicals and tyrosine derivatives in neutral media, which were characterized by essentially nonequilibrium kinetics.

One-Electron Redox Potentials of the Tryptophan and Tyrosine Derivatives. One-electron transfer equilibria of the indolyl and phenoxyl radicals having various side chains can be envisaged as follows:

$$R\text{–}IndH^{+}\cdot + A^- \rightleftharpoons R\text{–}IndH + A\cdot \quad (7)$$

$$R\text{–}Ind\cdot + A^- \rightleftharpoons R\text{–}Ind^- + A\cdot \quad (8)$$

$$R\text{–}Ind^- + H^+ \rightarrow R\text{–}IndH \quad (9)$$

$$pK_a > 14$$

$$R\text{–}PhO\cdot + A^- \rightleftharpoons R\text{–}PhO^- + A\cdot \quad (10)$$

where A^- and $A\cdot$ denote respectively reduced and oxidized forms of an electron donor. Providing that the ET equilibria 7, 8, or 10 are achieved in the rate-determining step, the redox potential difference between the following redox couples

$$R\text{–}Ind\cdot + H^+ + e^- \rightarrow R\text{–}IndH \quad (11)$$

or

$$R\text{–}PhO\cdot + e^- \rightarrow R\text{–}PhO^- \quad (12)$$

and

$$A\cdot + e^- \rightarrow A^- \quad (13)$$

can be calculated from the formula

$$\Delta E = 0.059 \log K \quad (14)$$

where K is the apparent equilibrium constant of redox equilibria 7, 8, or 10. If the one-electron redox potential of the $A\cdot/A^-$ couple is known, the redox potential of the investigated redox couple can be derived from the redox potential difference. The reference redox couples used in this study were the following secondary standards:[25] $SO_3^{2-}/SO_3^{-}\cdot$ with $E_{3.6} = 0.84$ V,[17] *p*-CH_3OPhO^-/*p*-$CH_3OPhO\cdot$ with $E_{7.5} = 0.6$ V and $E_{13} = 0.4$ V,[18] and $G^-/G\cdot$[26]

TABLE III: One-Electron Redox Potentials of Tryptophan and Tyrosine Derivatives, 20 °C

derivative	E,[a] V pH 3	pH 7	pH 13
tryptophan	0.94	0.64	0.56
tryptophanamide	0.94	0.68	0.62
tryptamine	0.97	0.64	0.56
N-acetyltryptophan	~0.8–0.87	0.69	
tryptophan methyl ester		0.68	
tryptophyl-alanine		0.67	
tyrosine			0.64
		(0.85)	(0.65)
tyrosine methyl ester		0.87	0.66
		(0.85)	(0.65)
N-acetyltyrosinamide			0.65
		(0.85)	(0.65)
alanyl-tyrosine		0.85	
		(0.85)	(0.65)

[a] Measured values of redox potentials, estimated to be accurate to ±0.02 V. The values of redox potentials calculated from the measured values and the known values of pK_a's of the molecules and radicals by using the formula 15 in text are given in parentheses.

with $E_7 = 0.91$ V.[19] The reactions of the indolyl and phenoxyl radicals with reference compounds were monitored at two wavelengths, whenever possible. The equilibrium constants were derived from kinetic treatment of the experimental data and absorbances of radicals at equilibrium (for details see ref 17), and the results are summarized in Tables II and III.

One-electron redox potentials of the tryptophan and tyrosine derivatives were found to be moderately high (0.5–1 V) in the pH range studied. Redox potentials of the tyrosine derivatives were higher than those of tryptophan derivatives and independent of the structure and overall charge of the side chain. The values of the redox potentials of the tyrosine derivatives determined at different pH values allow calculation of the pH dependence of the redox potentials. The following formula was used in the calculations[20]

$$E_i = E_0 + 0.059 \log (K_a + [H^+]) \quad (15)$$

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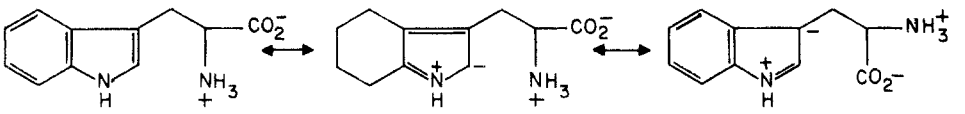
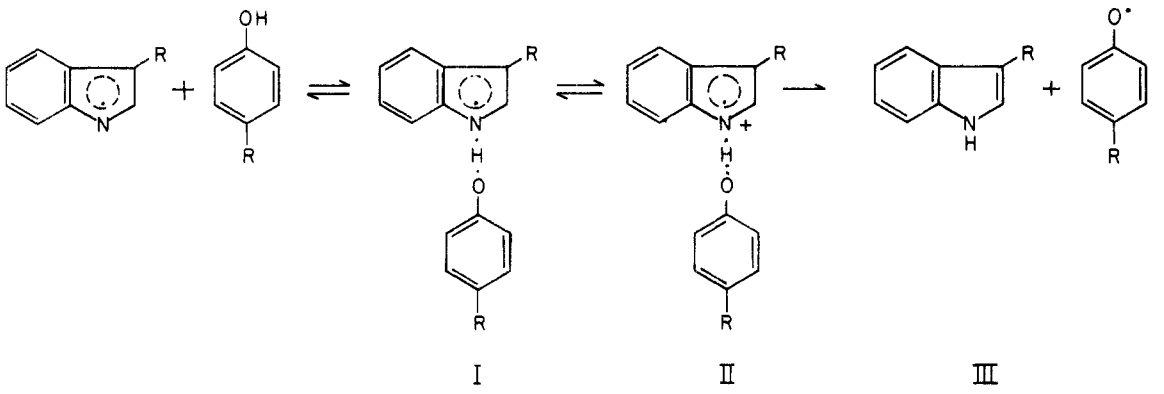
SCHEME I



SCHEME II



I II III

where E_i and E_0 respectively represent one-electron redox potentials at pH i and pH 0, K_a is the dissociation constant of phenol, and $[H^+]$ is the concentration of hydrogen ions.

One-electron redox potentials of tryptophan derivatives were found to depend on the structure and charge of the side chain. It can be seen from Table III that the difference in redox potentials of various tryptophan derivatives is most pronounced at pH 3 and 13, and amounts to ±0.1 V. This suggests that the properties of these redox couples may be associated with the ionizable groups in their side chains. In fact, it was found that electron transfer from tryptophan derivatives to the carbonate radical, $CO_3^{-\cdot}$, depended on the ionic strength of the medium in such way that the total charge on tryptophan derivatives was observed.[21] We observed no ionic strength dependence (μ = 0.1–5 M) for the rates of electron transfer from *p*-methoxyphenol to various indolyl radicals throughout the pH range 7.5–12.5. This shows that indolyl radicals behave as neutral species over a wide pH range, whereas the parent molecules exhibit the full electronic charge pertinent to that particular pH. Consequently, it might be suggested that dissociation constants of the ionizable groups in side chains of tryptophan derivatives differ from those in side chains of corresponding indolyl radicals. However, the acid–base properties of the side chain should cause minimum perturbation of the absorption spectrum of the indolyl radicals and cannot be readily measured by our techniques. This precludes meaningful evaluation of the pH dependence of the redox potentials of these redox couples.

The ability of the side chain to influence the redox properties of the tryptophan derivatives might be associated with formation of charged resonance forms, as shown in Scheme I. There are no activation barriers to rotation about the C_α–C_β bond in the side chain so that the above conformations are easily adopted. Any interaction between groups in the side chain and the pyrrole ring could result in preferred conformers and this might lower the redox potential for the couple.

Electron Transfer between Tryptophan and Tyrosine. Electron-transfer reactions between the indolyl radicals and tyrosine derivatives were studied in water solutions at pH 7.5, with azide radicals as primary oxidants. The overall reaction, monitored as the decay of the indolyl radicals at 525 nm and the buildup of phenoxyl radicals at 405 nm, can be envisaged as follows:

$$\text{R–Ind}\cdot + \text{R–PhOH} \rightarrow \text{R–IndH} + \text{R–PhO}\cdot \qquad (16)$$

The reactions of the indolyl radicals of various tryptophan derivatives with different tyrosine derivatives were found to proceed to completion, irrespective of the experimental conditions. The reaction rate constants are summarized in Table IV.

TABLE IV: Reactivities of the Indolyl Radicals with Tyrosine Derivatives, at pH 7.5, 20 °C, 0.1 M NaN_3

R–Ind· from	R–PhOH	k(R–Ind· + R–PhOH),[a] M^{-1} s^{-1}
alanyl-tryptophan	tyrosyl-alanine	4×10^5
tryptophan	tyrosine methyl ester	5.4×10^5
tryptamine	tyrosine methyl ester	4.8×10^5
tryptophyl-alanine	tyrosyl-alanine	1.1×10^6
tryptophyl-alanine	alanyl-tyrosine	1.3×10^6
tryptophyl-glycine	glycyl-tyrosine	1.3×10^6[b]

[a] Estimated to be accurate to ±20%. [b] From ref 4.

As can be seen from Table IV, the rates for ET from tyrosine derivatives to indolyl radicals were rather low, $k = 4 \times 10^5$–10^6 M^{-1} s^{-1}. However, these reactions went to completion in spite of the approximately 0.2-V barrier for electron transfer in that direction. Consequently, on the basis of the above premises, direct electron transfer between the R–Ind· radicals and R–PhOH can be ruled out. In order to explain the observed thermodynamically forbidden direct ET from tyr to trp., we propose the set of reactions shown in Scheme II. Formation of the intermediate complex, step I, might also be faciliated by the interaction of the side chains of the derivatives or the hydrophobic interactions in peptides and proteins. This step could provide a plausible explanation for the differences in the ET rates for various derivatives. Charge separation in step II may be viewed as a proton-transfer reaction which makes the actual electron-transfer step feasible. Either step I or II in Scheme II may be rate-determining, since the ET step is greatly favored by the redox potential difference of ~0.3 V between the indolyl radical cations and phenoxide ions. In fact, once the equilibria preceding the ET step are achieved, the redox reaction would pull the overall reaction to the right, since the equilibrium constant for II to III conversion can be estimated as $K \sim 10^5$.

Electron transfer from tyrosine to tryptophyl radicals was also studied in a trp-tyr dipeptide. In order to investigate the effect of the proton-donating ability of a solvent on the ET reaction, we generated tryptophyl radicals by $CCl_3O_2\cdot$ radicals in an air-saturated 2-propanol solution containing carbon tetrachloride and acetone (for details see ref 22). The rate of initial oxidation of the dipeptide was measured from the pseudo-first-order buildup of absorption at 450 nm, which is the isosbestic point of the initially produced spectrum of the indolyl radicals and the spectrum of the phenoxyl radicals generated in the subsequent ET reaction. The reactivity of $CCl_3O_2\cdot$ radicals with trp-tyr dipeptide, k = (1.7

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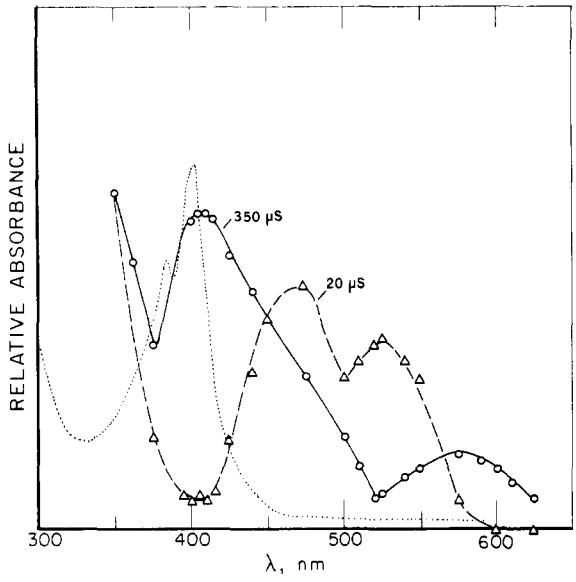

Figure 2. Transient spectra obtained upon $CCl_3OO\cdot$ radical reaction with tryptophanyl-tyrosine, 13.7 mM Trp-Tyr in air-saturated 2-propanol:acetone:CCl_4 (90:10:0.04 M) mixture, at 20 °C, 5 Gy. The experimental points represent the average of three absorbance readings obtained on successive pulsing. Broken line: spectrum taken upon completion of the initial oxidation at 20 μs. Full line: spectrum taken upon completion of secondary first-order process (see discussion) at 350 μs. Dotted line: spectrum of phenoxyl radicals in aqueous solution obtained upon oxidation of *N*-acetyltyrosinamide by the azide radical, given for comparison. 10 mM *N*-acetyltyrosinamide, 0.1 M NaN_3, pH 7.5, 30 Gy normalized to 5 Gy by assuming G(phenoxyl) = 5.4.

± 0.4) × 10^7 M^{-1} s^{-1}, is ~5 times slower than the corresponding rate of reaction with tryptophan in water, $k = 8.7 \times 10^7$ M^{-1} s^{-1}.[23] The electron transfer from tyrosine to tryptophyl radicals occurred in a subsequent, much slower first-order process with a rate constant of 13000 ● 4000 s^{-1}. This rate constant is considerably slower than that found in water, $k = 72000$ s^{-1},[2] which might be explained by the solvent effect on the proton-transfer reaction preceding electron transfer (see Scheme II). Figure 2 illustrates the changes in the optical absorption spectra obtained on initial reaction of $CCl_3O_2\cdot$ radicals with trp-tyr dipeptide and upon completion of electron transfer. The spectrum of the phenoxyl radicals produced by direct reaction of $CCl_3O_2\cdot$ radicals with the tyrosine derivatives could not be obtained since the rate of this reaction appeared to be unmeasurably slow, $k < 10^4$ M^{-1} s^{-1}. Hence, the spectrum of phenoxyl radicals generated by oxidation of *N*-acetyltyrosinamide with azide radicals at pH 7.5 is presented in Figure 2 for comparison.

As seen from Figure 2, the spectrum of transients obtained upon completion of the slower process is broader than the usual spectrum of tyrosine radicals. In addition, the absorption maximum is shifted from 405 to 425 nm and the spectrum features a low-intensity band at 590 nm which is not normally observed in the spectra of phenoxyl radicals. It could be suggested that the broadening of the phenoxyl spectrum and the presence of the 590-nm band is associated with the formation of a charge-transfer complex between the tryptophyl radicals and phenol in tyrosine, similar to II in Scheme II. This charge-transfer complex might be stabilized by strong hydrogen bonding in the less polar mixture of organic solvents, whereas its dissociation to phenoxyl radicals and tryptophan would be faciliated by water and/or proton acceptors or donors.

Electron transfer from tyrosine to tryptophyl radicals in trp-tyr dipeptide was also studied in aqueous solutions containing sodium azide at pH 6.0, where the catalysis of any proton-transfer reaction by hydrazoic acid, $pK_a = 4.7$,[3] could be investigated. The rate of decay of the initially produced tryptophyl radicals, which was the same as the rate of buildup of absorption characteristic of the phenoxyl radicals, was found to depend on the concentration of hydrazoic acid according to

$$k_{\text{overall}} = k_0 + k[HN_3] \qquad (17)$$

The rate constant for electron transfer in the uncatalyzed process was determined as k_0 (36000 ± 4000) s^{-1}, whereas the rate of the process catalyzed by hydrazoic acid was $k = (2.8 \pm 0.3) \times 10^6$ M^{-1} s^{-1}.

In view of these findings, we suggest that electron transfer from tyrosine to tryptophyl radicals in mixtures, peptides, and proteins proceeds through several intermediate complexes, one of which may involve a proton-transfer reaction. Proton transfer from phenol to tryptophyl radicals should be acid–base catalyzed and faciliated by the favorable conformations of the peptide or protein backbone.

Registry No. HSO_3^-, 14996-02-2; p-$MeOC_6H_4O^-$, 29368-59-0; L-tryptophan, 73-22-3; L-tryptophan methyl ester, 4299-70-1; DL-tryptophanamide, 7303-48-2; *N*-acetyl-L-tryptophan, 1218-34-4; *N*-L-tryptophyl-L-alanine, 24046-71-7; L-tyrosine, 60-18-4; *N*-acetyl-L-tyrosinamide, 1948-71-6; L-tyrosine methyl ester, 1080-06-4; L-tryptophan radical, 100927-20-6; DL-tryptophanamide radical, 100927-21-7; *N*-acetyl-L-tryptophan radical, 100927-23-9; tryptoamine radical, 100927-19-3; L-tryptophan methyl ester radical, 100927-22-8; *N*-L-tryptophyl-L-alanine radical, 100927-25-1; L-tyrosine radical, 16978-66-8; guanosine anion, 100927-93-3; *N*-acetyl-L-tyrosinamide anion, 53694-88-5; L-tyrosine methyl ester radical, 100927-94-4; *N*-L-alanyl-L-tyrosine radical, 100927-95-5; *N*-acetyl-L-tyrosinamide radical, 100927-96-6; *N*-L-alanyl-L-tryptophan radical, 100927-26-2; *N*-L-tryptophylglycine radical, 100927-24-0; *N*-glycyl-L-tyrosine, 658-79-7.

(23) Packer, J. E.; Mahood, J. S.; Willson, R. L.; Wolfenden, B. *Int. J. Radiat. Biol.* **1981**, *39*, 135–141.

(24) The mention of commerical products does not imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the products identified are necessarily the best available for the purpose.

(25) E_i are used to denote one-electron redox potentials of given redox couple at pH i.

(26) Produced on one-electron oxidation of guanosine with Tl(II) ions at pH 7.0.[19]