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Selectivity in the Reactions of e_{aq}^- and OH Radicals with Simple Peptides in Aqueous Solution. Optical Absorption Spectra of Intermediates

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Abstract: The reactivity and site of attack of OH radicals with the simple peptides acetylglycine, acetylglycylglycine, diglycine, and triglycine in aqueous solutions were studied using the technique of pulse radiolysis. The reaction rate constants k(OH + P) were found to be markedly dependent upon pH in the range 4–10 for Gly-Gly and Gly-Gly-Gly, but independent of pH for Ac-Gly and Ac-Gly-Gly. This dependence of k(OH + P) on pH was shown to correspond to the state of protonation of the terminal amino group. These results were used in the interpretation of the optical absorption spectra of the intermediates produced, *e.g.*, OH + $^+NH_3CH_2CONHCH_2COO^-$ → $^+NH_3CH_2CONH\dot{C}HCOO^-$ + H_2O; OH + $NH_2CH_2CONHCH_2COO^-$ → $NH_2\dot{C}HCONHCH_2COO^-$ + H_2O; OH + $CH_3CONHCH_2COO^-$ → $CH_3CONH\dot{C}HCOO^-$ + H_2O. The acid–base properties of the radicals were followed and pK values derived. The radicals Ac-NHĊHCOO$^-$ and Ac-Gly-NHĊHCOO$^-$ have absorption maxima in the region 250–270 nm and extinction coefficients ϵ ~14,000 M^{-1} cm^{-1}; the radicals $^+H_2$-Gly-NHĊHCOO$^-$ and $NH_2\dot{C}HCO$-Gly-O$^-$ (and their triglycine equivalents) have maxima at ~260 nm and ϵ values of ~12,000 and ~5000 M^{-1} cm^{-1}, respectively. At biological pH values, products from attack at both positions can be expected for the decomposition of these simple peptides. High selectivity for the reaction of e_{aq}^- with Gly-Gly and Gly-Ala has been observed, leading to almost quantitative (~80%) reductive deamination: e_{aq}^- + $^+NH_3CH_2CONHCHRCOO^-$ → NH_3 + $\cdot CH_2CONHCHRCOO^-$. The $\cdot CH_2CONHCHRCOO^-$ radicals have maxima at ~435 nm and extinction coefficients of ~1200 M^{-1} cm^{-1}.

Some of the major functional groups in proteins are peptide linkages, –CONH–, amino, carboxyl, –S–S–, and –SH groups. In a recent article, the radiation chemistry of aqueous solutions of some of these compounds was reviewed by Garrison.[2] A systematic study using the technique of pulse radiolysis is presently being employed as a complementary and more direct probe to identify the sites of attack by e_{aq}^- and OH radicals on the molecular structure of these compounds. Up to date, the pulse radiolysis of aqueous solutions of some aliphatic acids,[3] amides,[4] aliphatic[5] and aromatic[6] amino acids, and sulfur compounds[7] has been investigated.

From the radiation chemistry of acetylglycine in aqueous solutions, it was shown[2] that the OH radicals attack the peptide methylene group in preference to the end methyl group

$$OH + CH_3CONHCH_2COO^- \longrightarrow CH_3CONH\dot{C}HCOO^- + H_2O \quad (1)$$

(1) National Academy of Science National Research Council Research Associates at Natick.
(2) W. M. Garrison, *Curr. Top. Radiat. Res.*, 4, 43 (1968).
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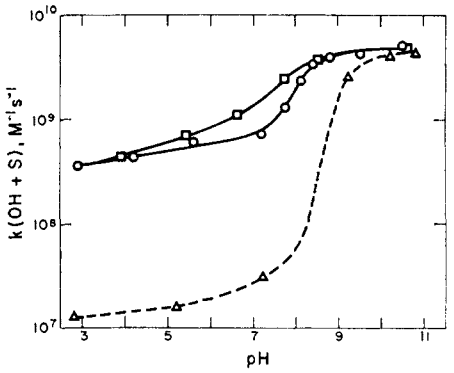

Figure 1. Dependence upon pH of the overall rate constant for reaction of OH radicals with glycine, diglycine, and triglycine. Data for glycine are derived from ref 14.

The radicals predominantly dimerize, since the major product is the α,α-N-acetyldiaminosuccinic acid derivative ($G \simeq 1.6$ at pH 3.0, O_2-free)

$$2CH_3CONHCHCOO^- \longrightarrow \begin{matrix} CH_3CONHCHCOO^- \\ | \\ CH_3CONHCHCOO^- \end{matrix} \quad (2)$$

Disproportionation takes place to a smaller extent, and is presumed to lead to the formation of the dehydropeptide. Other peptides, such as diglycine, have been assumed[8] to undergo similar reactions with the OH radicals. In systems containing O_2 the radicals rapidly react with O_2, yielding peroxy radicals, and leading eventually to cleavage of the peptide bond.

While the rate of reaction of e_{eq}^- with the acetyl derivatives of aliphatic amino acids is relatively slow, *e.g.*, $k(e_{eq}^- + \text{Ac-Gly}) \sim 10^7\ M^{-1}\ \text{sec}^{-1}$ at pH 6, the reaction of e_{eq}^- with peptides in their zwitterionic form is much faster, with $k \geq 3 \times 10^8\ M^{-1}\ \text{sec}^{-1}$

$$e_{eq}^- + \overset{+}{N}H_3CH_2CONHCH_2COO^- \longrightarrow NH_3 + \cdot CH_2CONHCH_2COO^- \quad (3)$$

In the presence of OH radical scavengers, the yield of reductive deamination *via* reaction 3 was found to be $G(NH_3) = 2.5$. Deamination of simple peptides was also observed by the esr technique on radiolysis of peptides in the solid state.[9]

In this work the mechanism of reaction of OH radicals with acetylglycine (Ac-Gly), acetylglycylglycine (Ac-Gly-Gly), diglycine (Gly-Gly), and triglycine (Gly-Gly-Gly) as well as the spectral, acid–base, and kinetic properties of the resulting radicals were studied. From the correlation of the rates, k(OH + P), and the spectra of the free-radical intermediates as a function of pH, selectivity in the site of attack of OH radicals on these simple peptides was found. In addition, a spectral method for the study of reductive deamination of peptides by e_{eq}^- was developed.

Experimental Section

A Febetron (Field Emission Corp.) 705 pulsed radiation source, producing an electron beam of 2.3-MeV energy and single pulses of ~30-nsec duration, was used in this work. Full experimental details with respect to the pulsed xenon lamp light source, double monochromators, dosimetry, and water purification have been described elsewhere.[10] Analytical reagent grade chemicals were supplied by Calbiochem and Cyclochemical Corp. Solutions were buffered using perchloric acid, potassium hydroxide, sodium tetraborate (2–5 mM), and potassium phosphate (~1–3 mM). Doses of 2.4–20 krads/pulse were used and were derived on the basis of $g(e_{aq}^-) = g(\text{OH}) = 2.8$. Owing to the relatively high dose per pulse, the concentration of solutes was chosen on the basis of known radical–radical and radical–solute rate constants.[11] The OD values were read at ~1 μsec and extrapolated to zero time.

Results

The hydrated electrons and OH radicals, generated in the radiolysis of water, react readily with peptides

$$H_2O \text{ —}\sim\!\!\!\sim\!\!\!\longrightarrow e_{aq}^-, \text{OH, H, } H_2, \text{ and } H_2O_2$$

These reactions were studied separately under well-controlled conditions. The OH radical reactions were examined in N_2O-saturated solutions (1 atm, $[N_2O] \sim 2.5 \times 10^{-2}\ M$) when the e_{aq}^-'s are converted ($\geq 98\%$) into OH radicals, according to reaction 4, where $k_4 \sim$

$$e_{aq}^- + N_2O \longrightarrow N_2 + \text{OH} + \text{OH}^- \quad (4)$$

$6 \times 10^9\ M^{-1}\ \text{sec}^{-1}$.[11] Under these conditions virtually all the reactive species are OH radicals. The H atoms (~12%) initially produced are expected to react with these solutes, similar to the OH radicals; their contribution was taken into account. The e_{aq}^- reactions were examined in presence of excess tertiary alcohols (*t*-BuOH or *t*-AmOH) to scavenge the OH radicals. The transients produced from these alcohols have low extinction coefficients above 250 nm, and corrections were made in all cases for their absorption.

Reactions of OH Radicals. Determination of k(OH + Peptides). The rates of reaction of OH radicals with the peptides were determined in the presence of N_2O (1 atm) using the potassium thiocyanate method.[12,13] The OH radicals react with CNS^- ions to produce $\cdot(CNS)_2^-$ radical anions with λ_{max} 500 nm. The intermediates produced from the reaction of OH radicals with the peptides do not contribute to the absorption at 500 nm under these conditions. The ratios of reaction rates were determined from

$$\frac{k(\text{OH} + \text{P})}{k(\text{OH} + \text{CNS}^-)} = \frac{\text{OD}}{\text{OD}_0 - \text{OD}} \frac{[\text{P}]}{[\text{CNS}^-]}$$

where OD_0 is the absorption at [P] = 0. The concentration of CNS^- was kept constant at 2 mM, while the concentration of the peptides was varied up to 0.1 M. The absolute rates were calculated taking $k(\text{OH} + \text{CNS}^-) = 1.1 \times 10^{10}\ M^{-1}\ \text{sec}^{-1}$.

The variation with pH in the rate constant k(OH + S) for diglycine and triglycine is shown in Figure 1; in addition, the k(OH + glycine) values[14] are shown for comparison. The rates of OH with the protonated forms of $^+H_2$-Gly-Gly and $^+H_2$-Gly-Gly-Gly were measured[14] and are in general agreement with those given here; however, no rates were determined for the reaction of OH radicals with the deprotonated peptides. The large increase in the reactivity of OH radicals with increasing pH can be seen to correspond with

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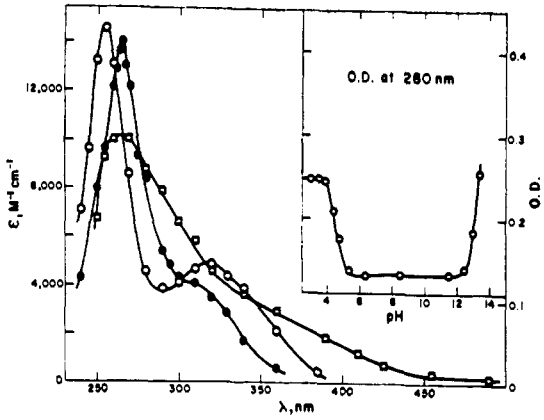

Figure 2. Absorption spectra of intermediates produced on pulse radiolysis of 0.04 M N-acetylglycine in the presence of N_2O (1 atm), dose = 2.4 krads/pulse: at pH 3.0, ●; pH 8.5, ○; pH 13.4, □. Insert: OD_{280} *vs.* pH curve of transient.

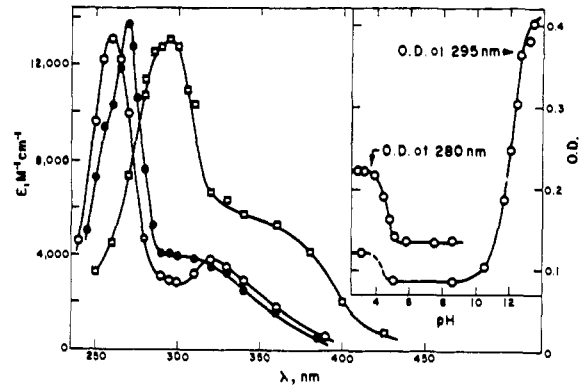

Figure 3. Absorption spectra of intermediates produced on pulse radiolysis of 0.01 M N-acetylglycylglycine in the presence of N_2O (1 atm), dose = 2.4 krads/pulse: at pH 3.2, ●; pH 8.6, ○; pH 13.2, □. Insert: OD *vs.* pH curve of transient monitored at 280 and 295 nm.

the pK for deprotonation of the amino groups of these compounds. A similar deactivation of α hydrogens by $-NH_3^+$ groups, toward OH radical attack, has been observed for some longer chain amino acids (*e.g.*, α-aminobutyric acid, valine). The actual k values for the different ionic forms of the peptides investigated are presented in Table I.

Table I. Rates of Reaction of OH Radicals with Simple Peptides, at Various pH Values, in Aqueous Solution

Solute	pH	Ionic form	k(OH + S), M^{-1} sec^{-1}
Glycine	1.0	$^+H_2$-Gly-OH	1.6×10^7 [a]
	5.2	$^+H_2$-Gly-O$^-$	1.6×10^7 [b]
	10.8	H-Gly-O$^-$	5.0×10^9 [b]
Diglycine	4.2	$^+H_2$-Gly-Gly-O$^-$	4.4×10^8
	10.5	H-Gly-Gly-O$^-$	5.2×10^9
Triglycine	5.4	$^+H_2$-Gly-Gly-Gly-O$^-$	7.3×10^8
	10.6	H-Gly-Gly-Gly-O$^-$	5.0×10^9
Acetylglycine	8.7	Ac-Gly-O$^-$	4.2×10^8
Acetylalanine	9.2	Ac-Ala-O$^-$	4.6×10^8
Acetylglycylglycine	8.6	Ac-Gly-Gly-O$^-$	7.8×10^8

[a] Reference 11. [b] Reference 14.

Optical Absorption Spectra of Intermediates. The transient absorption spectra which result from the reaction of OH radicals with Ac-Gly were found to be strongly dependent on pH over certain pH regions (see Figure 2). In acidic solutions, at pH <4, the absorption maximum is at 265 nm with a high extinction coefficient, ϵ_{265} 14,000 M^{-1} cm^{-1} (see Table II). A shoulder at ~310 nm indicates the presence of either another transient or another absorption band of the same transient. At pH 6.0 a shift of ~10 nm in the maximum takes place and the small shoulder now becomes a pronounced peak at ~320 nm. The decay rates of both peaks have been found to be identical, however, supporting the notion of only one transient with two absorption bands. Above pH 12 another change in the spectrum takes place. The absorption in these alkaline solutions shows a mixed decay, which was not resolved.

The reaction of OH radicals with Ac-Gly-Gly results in spectra very similar to those of the Ac-Gly transients Figure 3). Here again the first change takes place between pH 3.8 and 6.0, with the same pK as for the Ac-Gly transients (pK 4.5). The second change takes place above pH 10; this occurs at a considerably lower pH than for the Ac-Gly transient. The OD *vs.* pH curve indicates the beginning of a plateau around pH 13.5.

The spectra of the intermediates produced from the reaction of OH radicals with Gly-Gly and Gly-Gly-Gly are shown in Figures 4 and 5. Although they demonstrate certain similarities with the spectra of the transients from Ac-Gly and Ac-Gly-Gly, the nature of the intermediates and their dependence upon pH are quite different. At low pH the main peaks for Gly-Gly and Gly-Gly-Gly are at 260 and 263 nm, respectively, with high ϵ values, although not as high as those of the acetyl derivatives. From the change of OD with pH at a fixed wavelength (see inserts in Figures 4 and 5), two overlapping curves representing two different changes are considered to be present in the pH range 4–9. These two overlapping changes are assumed to represent proton dissociation from the carboxyl groups of the corresponding radicals and change in the site of attack by OH radicals (see below). Exact pK values cannot be derived under these conditions, yet approximate values can be obtained if one assumes that the first change covers the same pH range as for the acetyl derivatives, namely dissociation of the carboxyl groups. These approximate pK values are listed in Table II.

Above pH ~11.0, another change occurs in the absorption spectra and extinction coefficients of the transients. The nature of this change will be discussed below.

Reactions of e_{aq}^-. In neutral solutions, the acetyl derivatives of the simple peptides studied have a considerably lower reactivity toward e_{aq}^- ($k \sim 10^7$ M^{-1} sec^{-1}) than their counterparts when the amino group is protonated. The reaction rate[11] of $^+H_2$-Gly-Gly-O$^-$ and $^+H_2$-Gly-Ala-O$^-$ toward e_{aq}^- is $\sim 3 \times 10^8$ M^{-1} sec^{-1}. Using an appropriate concentration of OH radical scavengers, it was possible, under our experimental conditions, to observe the intermediates pro-

Table II. Absorption Maxima, Extinction Coefficients, Decay Kinetics, and p*K* Values of Intermediates Produced from the Reaction of OH Radicals with Simple Peptides in Aqueous Solution

Solute	pH	λ_{max}, nm	ϵ, M^{-1} cm^{-1}	$2k$, M^{-1} sec^{-1}	Suggested radical	pK_a Radical	pK_a Solute -COOH	pK_a Solute $-NH_3^+$
Acetylglycine	3.0	265, 310	14000, ~3900	1.1×10^9	Ac-NHĊHCOOH			
					⇅	4.6	3.7	
	8.5	255, 320	14000, 4800	8.0×10^8, 7.0×10^8	Ac-NHĊHCOO$^-$			
	13.4	265	10000			≥13[a]		
Acetylglycyl-glycine	3.2	270, 310	13700, ~3900	6.7×10^8	Ac-Gly-NHĊHCOOH			
					⇅	4.5	3.6	
	8.6	260, 320	13000, ~3700	2.6×10^8, 2.4×10^8	Ac-Gly-NHĊHCOO$^-$			
	13.2	295	13000			~12[a]		
Diglycine	2.8	260, 310	11500, 3000	1.4×10^9	$\overset{+}{H_2}$-Gly-NHĊHCOOH			
					⇅	~5	3.06	
	~6.0			6.3×10^8	$\overset{+}{H_2}$-Gly-NHĊHCOO$^-$			
					↓	~7[a]		8.1
	10.0	260	4700	4.5×10^8	NH$_2$ĊHCO-Gly-O$^-$			
	13.5	260	8700			>12.6[a]		
Triglycine	3.8	263, 340	12900, 3500	6.2×10^8	$\overset{+}{H_2}$-Gly-Gly-NHĊHCOOH			
					⇅	~5	3.26	
	~6.0			5.0×10^8	$\overset{+}{H_2}$-Gly-Gly-NHĊHCOO$^-$			
					↓	~7[a]		7.9
	8.9	265	6500	4.5×10^8	NH$_2$ĊHCO-Gly-Gly-O$^-$			
	13.3	285	9200			>13.3[a]		

[a] These are derived pH values for 50% change in the nature of the intermediates.

duced from the reaction of e_{aq}^- with Gly-Gly and Gly-Ala.

Figures 6 and 7 show that the transient spectra produced from the reaction of e_{aq}^- with Gly-Gly and Gly-Ala at pH ~7.0 have maxima at λ ~435 nm. In the presence of N_2O (1 atm), these absorption maxima

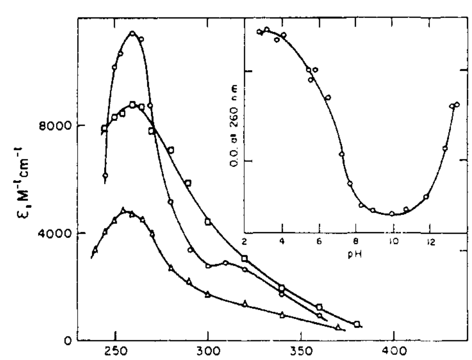

Figure 4. Absorption spectra of intermediates produced on pulse radiolysis of 0.1 *M* diglycine in the presence of N_2O (1 atm), dose = 2.4 krads/pulse: at pH 2.8, ○; pH 10, △; pH 13.5, □. Insert: OD_{260} *vs.* pH curve of transient.

disappear, which is as expected, since under these conditions >95% of the e_{aq}^-'s should react with N_2O. The residual absorptions below 300 nm (Figures 6 and 7) are probably due to partial reaction of H atoms and OH radicals with the peptides.

Since the reaction of e_{aq}^- with the chloroacetyl derivatives of glycine and alanine is expected[3] to lead to quantitative dechlorination, these compounds were irradiated in presence of 1.0 *M* *t*-BuOH to observe the absorption spectra and determine the extinction coefficients of the $\cdot CH_2CO$-Gly-O$^-$ and $\cdot CH_2CO$-Ala-O$^-$ radicals

$$e_{aq}^- + ClCH_2CONHCH_2COO^- \longrightarrow \dot{C}H_2CONHCH_2COO^- + Cl^- \quad (5)$$

$$e_{aq}^- + ClCH_2CONHCH(CH_3)COO^- \longrightarrow \dot{C}H_2CONHCH(CH_3)COO^- + Cl^- \quad (6)$$

The equivalence of these radicals with the transients produced from the reaction of e_{aq}^- with Gly-Gly and Gly-Ala confirms the proposed reductive deamination which these peptides undergo on reaction with e_{aq}^-. In

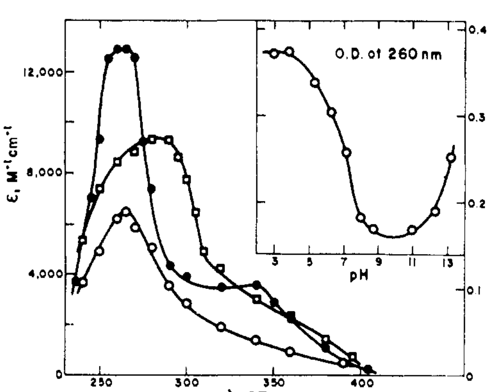

Figure 5. Absorption spectra of intermediates produced on pulse radiolysis of 0.01 *M* triglycine in the presence of N_2O (1 atm), dose = 2.4 krads/pulse: at pH 3.8, ●; pH 8.9, ○; pH 13.3, □. Insert: OD_{260} *vs.* pH curve of transient.

addition, the efficiency of deamination was obtained on the basis of the extinction coefficients derived from the chloroacetyl compounds. The per cent deamination is given in Table III.

The decay rates of the deaminated radicals are of the order of ~2×10^9 M^{-1} sec^{-1} (see Table III). It is possible that a fraction of these radicals decays by

Table III. Absorption Maxima, Extinction Coefficients, Efficiency of Deamination, and Decay Kinetics of Intermediates Produced from the Reaction of e_{aq}^- with Simple Peptides

Solute	pH	λ_{max}, nm	Suggested radical	ϵ, M^{-1} cm^{-1}	$2k$, M^{-1} sec^{-1}	% deamination
Cl-Ac-NH_2[a]	7.0	400	$\cdot CH_2CONH_2$	1100	3.0×10^9 [b]	
Cl-Ac-Gly-O^-	8.5	435	$\cdot CH_2CO$-Gly-O^-	1150		
H_2-Gly-Gly-O^-	7.0	435	$\cdot CH_2CO$-Gly-O^-		1.8×10^9 [b]	80
Cl-Ac-Ala-O^-	8.5	435	$\cdot CH_2CO$-Ala-O^-	1200		
H_2-Gly-Ala-O^-	7.5	435	$\cdot CH_2CO$-Ala-O^-		1.5×10^9	75

[a] From ref 4. [b] Obtained in the presence of 1 M t-BuOH (as an OH radical scavenger), using ϵ values derived from corresponding chloro derivatives.

reaction with the t-BuOH radical formed under these conditions.

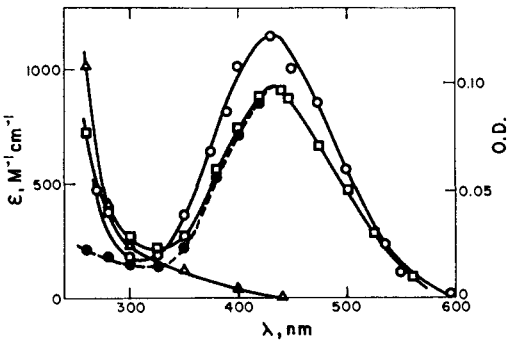

Figure 6. Absorption spectra of intermediates produced on pulse radiolysis in the presence of 1.0 M t-AmOH and argon (1 atm): 0.02 M diglycine, pH 7, □; 0.02 M N-chloroacetylglycine, pH 8.5, ○. Δ represents results for Gly-Gly in the presence of N_2O (1 atm), and ● the difference in absorption between OD_{Ar} and $\frac{1}{2}(OD_{N_2O})$. Dose = 19 krads/pulse.

Discussion

In the study of the reactions of e_{aq}^- and OH radicals with molecules containing the peptide group –CO–NH–, there are two classes of compounds which need to be discussed separately: (a) the peptides with an amino group in the terminal position, and (b) the acyl derivatives, lacking that amino group. The selectivity in the sites of attack by e_{aq}^- and OH radicals on peptides is markedly dependent upon the presence of the amino group and the state of its protonation.

The site of attack of the OH radical on the acetyl derivatives can be inferred from the reactivities of the related compounds. For instance, in the case of Ac-Gly, the attack on the end methyl group is expected to be considerably less favorable than on the methylene group, *i.e.*, $k_1 > k_7$.

$$OH + CH_3CONHCH_2COO^- \longrightarrow \cdot CH_2CONHCH_2COO^- + H_2O \quad (7)$$

$$OH + CH_3CONHCH_2COO^- \longrightarrow CH_3CONH\dot{C}HCOO^- + H_2O \quad (1)$$

From the results obtained in the determination of the site of attack of OH radicals in simple amides,[4] it was established that the amide nitrogen activates N-methyl groups, such that hydrogen abstraction from the N-methyl group is an order of magnitude (or more) faster than from the α-methyl group, *e.g.*

$$OH + CH_3CONH_2 \longrightarrow \dot{C}H_2CONH_2 + H_2O \quad (8a)$$

$$OH + CH_3CONHCH_3 \longrightarrow CH_3CONH\dot{C}H_2 + H_2O \quad (8b)$$

The predominance of reaction 1 observed in this work is also in agreement with the products determined from the γ radiolysis of aqueous solutions of Ac-Gly.

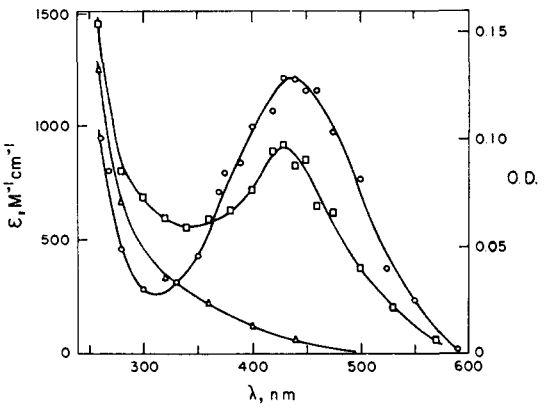

Figure 7. Absorption spectra of intermediates produced on pulse radiolysis in the presence of 1.0 M t-BuOH and argon (1 atm): 0.025 M glycylalanine, pH 7.5, □; 0.025 M N-chloroacetylalanine, pH 8.5, ○. Δ shows results for Gly-Ala in the presence of N_2O (1 atm). Dose = 19 krads/pulse.

The observed change in the absorption spectra of the transients in the pH range 4–6, Figure 2, is similar to the acid–base equilibrium previously observed[3] for a number of other carboxy radicals

$$CH_3CONH\dot{C}HCOOH \underset{}{\overset{pK_a\,4.6}{\rightleftharpoons}} CH_3CONH\dot{C}HCOO^- + H^+ \quad (9)$$

This pK value is higher than the pK value of the parent compound (pK_a = 3.67), indicating again the ability of the unpaired electron in the α position to the carboxyl group to decrease the acidity of the carboxyl group.

A similar reasoning and interpretation are found to apply for the intermediates produced from the reaction of OH radicals with Ac-Gly-Gly, Figure 3. From the pK value obtained for deprotonation of the carboxyl group of the transient (see Table II), it seems that the unpaired electron is in the α position to the carboxyl group, Ac-Gly-NHĊHCOO$^-$. There is at present no direct evidence to support abstraction from the –NH–CH_2–CO– group, *e.g.*, the formation of Ac-NHĊHCO-Gly radicals from Ac-Gly-Gly. The absorption spectra of both types of radical could, however, be quite similar.

The change in the transient spectra in alkaline solutions of Ac-Gly and Ac-Gly-Gly cannot be related to the dissociation of the OH radical

$$OH + OH^- \overset{pK\ 11.9}{\rightleftharpoons} O^- + H_2O$$

since the changes are observed (Figures 2 and 3) at different pH values. One could speculate that these changes may be related to a possible enolization of the peptide group

$$-CONH- \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} -C(OH)=N-$$

and a change in the reaction mechanism (*e.g.*, addition of OH or O^- to the double bond). Alternatively, the peptide hydrogen nearest to the free electron may undergo acid–base reactions. The onset of this change is at lower pH for Ac-Gly-Gly than for Ac-Gly, possibly due to a lowering of the pK in conjugated peptide groups. It is hoped that further work will clarify the results in this pH region.

As emphasized above, the presence of terminal amino groups, as in Gly-Gly and Gly-Gly-Gly considerably affects the reactivity and the site of attack by OH radicals. Since the reactivity of Gly-Gly and Gly-Gly-Gly at pH ~5.0 is 20–40 times that of glycine, Table I, one can predict that the OH radicals do not attack the $^+NH_3$–CH_2– groups but rather abstract from the –$NHCH_2COO^-$ and perhaps the –$NHCH_2CO$– groups. The deprotonation of the carboxyl groups of monofunctional radicals (*e.g.*, simple acids[3] and the acetyl derivatives presented above) is usually a simple sigmoidol OD *vs.* pH curve, but a more complex curve is obtained for Gly-Gly and Gly-Gly-Gly (see inserts of Figures 4 and 5) in the pH range 3–9. In addition to the acid–base equilibria

$$H_2{}^+\text{-Gly-NH}\dot{C}\text{HCOOH} \overset{pK\sim5.0}{\rightleftharpoons} H_2{}^+\text{-Gly-NH}\dot{C}\text{HCOO}^- + H^+ \quad (10)$$

$$H_2{}^+\text{-Gly-Gly-NH}\dot{C}\text{HCOOH} \overset{pK\sim5.0}{\rightleftharpoons} H_2{}^+\text{-Gly-Gly-NH}\dot{C}\text{HCOO}^- + H^+ \quad (11)$$

a change in the site of attack is proposed to take place with increasing pH. Since deprotonation of the $-NH_3^+$ groups increases the overall reactivity not only of glycine but of Gly-Gly and Gly-Gly-Gly (Figure 1), the deprotonated amino group now activates the α-CH_2 group for abstraction by OH radicals

$$OH + NH_2CH_2CO\text{-Gly-O}^- \longrightarrow NH_2\dot{C}HCO\text{-Gly-O}^- + H_2O \quad (12)$$

$$OH + NH_2CH_2CO\text{-Gly-Gly-O}^- \longrightarrow NH_2\dot{C}HCO\text{-Gly-Gly-O}^- + H_2O \quad (13)$$

The pH at which this change takes place is dependent on (a) the pK of the parent compound and (b) the difference in the rate constants for reaction of OH radicals with the protonated and deprotonated amino form of the peptides. For example, in 0.2 M Gly-Gly, about 0.5% or 10^{-3} M of the peptide is present in the deprotonated form at pH 6.0. However, k(OH + $^+H_2$-Gly-Gly-O^-)/k(OH + H-Gly-Gly-O^-) ~ 12, therefore about 6% of the OH radicals already reacts *via* reaction 12 at this pH. Although the initially formed radical is $NH_2\dot{C}HCO$-Gly-O^-, subsequent equilibration at these pH's

$$NH_2\dot{C}HCO\text{-Gly-O}^- \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} {}^+NH_3\dot{C}HCO\text{-Gly-O}^-$$

could take place. Hence, this change in the site of attack at near-biological pH's is expected to lead to significant changes in the nature of the products produced from the decomposition of peptides and proteins.

The spectral features of the $^+H_2$-Gly-$NH\dot{C}HCOO^-$ radicals (two absorption bands and high extinction coefficients) are very similar to those of the radicals observed from the acetyl derivatives (*e.g.*, $CH_3CONH\dot{C}HCOO^-$) and from amides (*e.g.*, $CH_3CONH\dot{C}H_2$), and differ substantially from those of the $NH_2\dot{C}HCO$-Gly-O^- radicals. It is conceivable that with increasing length of the peptides and/or with an increasing reactivity of the side chains, the fraction of these radicals will diminish and radicals from the side chain or –$NH\dot{C}HCO$– radicals may be produced to a greater extent.

The changes observed for Gly-Gly and Gly-Gly-Gly in alkaline solutions, Figures 4 and 5, may be similar to those suggested above for the acetyl derivatives.

Reductive deamination has been shown to take place on reaction of e_{aq}^- with Gly-Gly and Gly-Ala in aqueous solutions, with the formation of the corresponding acetyl, $\cdot CH_2CO$–, radicals

$$e_{aq}^- + {}^+NH_3CH_2CONHCH_2COO^- \longrightarrow NH_3 + \cdot CH_2CONHCH_2COO^- \quad (3)$$

From the determined extinction coefficients for these radicals, it was established that 80 and 75% of the e_{aq}^-'s deaminate Gly-Gly and Gly-Ala. These acetyl radicals have characteristic absorption maxima at ~435 nm and extinction coefficients of ~1200 M^{-1} cm^{-1}. The esr spectra of some of these radicals have recently been observed[15] in irradiated aqueous solutions, and confirm the pulse radiolysis results mentioned above. The rest of the e_{aq}^-'s may be converted to H atoms or add to carbonyl groups, *e.g.*

$$e_{aq}^- + {}^+NH_3CH_2CONHCH_2COO^- \longrightarrow H + NH_2CH_2CONHCH_2COO^-$$
$$\xrightarrow{+H_2O} {}^+NH_3CH_2\dot{C}(OH)NHCH_2COO^- + OH^-$$

(15) P. Neta and R. W. Fessenden, *J. Phys. Chem.*, 74, 2263 (1970).