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FREE RADICAL INTERMEDIATES PRODUCED IN THE PULSE RADIOLYSIS
OF SIMPLE PEPTIDES IN AQUEOUS SOLUTION

by

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I. INTRODUCTION

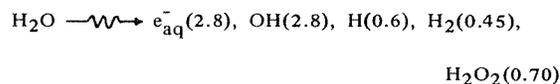
The biochemical importance of proteins, both as structural material and as catalysts of physiological reactions, is such that they can be said to be intimately associated with all the fundamental manifestations of life. The biological activity (enzymatic, hormonal, or immunological) of a protein molecule is often destroyed by relatively small doses of ionizing radiations, and the physico-chemical properties are extensively altered due to damage revealed in the amino acid side-chains, the production of new groups, the splitting of peptide

and hydrogen bonds, and the formation (or rupture) of both inter- and intramolecular crosslinks — some of these effects leading to conformational changes. Some of the major functional groups in proteins are amino groups, peptide linkages (-CONH-), carboxyl groups, sulfhydryl (-SH) and -S-S-, and aromatic groups. This article will deal with various aspects of the radiation chemistry of the essential functional groups in peptide chemistry, *viz.*, the -NH_3^+ , -CONH- , and -COO^- groups.

II. BASIC PRINCIPLES OF RADIATION CHEMISTRY

The absorption of energy (ionizing radiations) in radiation chemistry arises from the coulombic interactions of a fast charged particle and the electrons of the molecules of matter. As a result of these interactions, energy can be transferred from the fast charged particle to the electrons of molecules, raising the electrons from the ground state to some excited or ionized state. In radiation chemistry, therefore, the primary energy is non-selectively absorbed since it is essentially on the basis of the mass of the medium, and ionization as well as excitation processes always occurs. In contrast, in photochemistry the energy is selectively absorbed, and electronic excitation is by far the main consequence of absorption of light. In certain instances in photochemistry, ionization can also occur when the energy of the exciting light exceeds the ionization potential of the molecule or when the light energy absorption corresponds to a charge-transfer process (*e.g.*, in C.T.T.S. or C.T.T.L.).

The absorption of ionizing radiations by water and aqueous solutions leads to the formation of solvated electrons, hydroxyl radicals, and H atoms, in addition to the molecular products H_2 and H_2O_2 :



where the number in brackets represents *G*-values, *i.e.*, the number of radicals or molecules formed per 100 eV of absorbed energy. Although the formation of these primary species is an extremely fast process (*e.g.*, the hydration of e^- to e_{aq}^- is over in $< 10^{-11}$ sec and the reaction $\text{H}_2\text{O}^+ + \text{H}_2\text{O} \longrightarrow \text{OH} + \text{H}_2\text{O}^+$ in $\sim 10^{-11}$ sec), the lifetime of these radicals depends on the concentration and the reactivity of the solutes (or impurities) in the water.

III. EXPERIMENTAL TECHNIQUE

The transient optical absorption spectra of the intermediates produced from the reaction of e_{aq}^- and OH radicals with simple peptides in aqueous solution were obtained using the technique of pulse radiolysis. Using electron-pulse generators which provide single pulses of electrons of short dura-

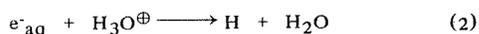
tion (30 nanoseconds in this case), it is possible to observe the changes in optical transmission of the solution immediately after the pulse by kinetic absorption spectrophotometry methods. The experimental technique and details used in this work have been described elsewhere.¹

The hydrated electron e_{aq}^- is a strongly absorbing species with $\lambda_{\text{max}} \sim 710$ nm at 25°C and $\epsilon_{710} = 1.8 \times 10^4 M^{-1} \text{cm}^{-1}$. In comparison, the OH radical and H atoms have low extinction coefficients ($< 1000 M^{-1} \text{cm}^{-1}$) and start absorbing below ~ 250 nm. Under certain conditions, it is possible to interconvert some of these species. For instance, e_{aq}^- can be converted to OH radicals on reaction with nitrous oxide (1 atm $\sim 2.5 \times 10^{-2} M$):



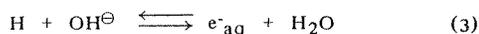
with $k_1 = 6 \times 10^9 M^{-1} \text{sec}^{-1}$ (ref 2). Hence the reactions of OH radicals can be studied (in presence of N_2O) without interference from e_{aq}^- , since both N_2O and N_2 are otherwise relatively inert.

Similarly, e_{aq}^- can be converted to H atoms in acidic solutions:



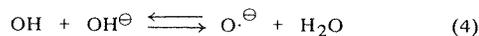
$$k_2 = 2.5 \times 10^{10} M^{-1} \text{sec}^{-1} \text{ (ref 2)}$$

In alkaline solutions, the following reactions occur:



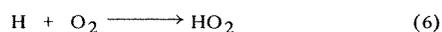
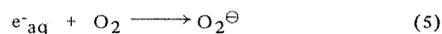
$$k_3 = 2.0 \times 10^7 M^{-1} \text{sec}^{-1} \text{ (ref 2)}$$

and



$$pK_4 = 11.9 \text{ and } k_4 \sim 5 \times 10^8 M^{-1} \text{sec}^{-1}$$

Oxygen is a very reactive gas and reacts by almost diffusion-controlled rate with e_{aq}^- and H atoms:



$$k_5 = 2.0 \times 10^{10} M^{-1} \text{sec}^{-1}$$

$$k_6 = 2.0 \times 10^{10} M^{-1} \text{sec}^{-1}$$

$$pK_7 \sim 4.8$$

For more detailed information on the radiation chemistry of aqueous solutions and the technique of pulse radiolysis, recently published books³⁻⁵ can be referred to.

IV. PULSE RADIOLYSIS OF SIMPLE PEPTIDES

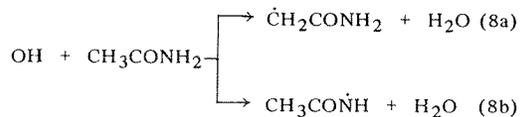
The radiation chemistry of simple peptides in aqueous solution has recently been reviewed by Garrison.⁶ Based on the nature of the products produced, mechanisms were proposed for the reactions of e_{aq}^- and OH radicals with the various peptides examined. The primarily-formed intermediates were not, however, observed or identified.

In order to study the reactions of e_{aq}^- and OH radicals with peptides and elucidate the effect of the characteristic functional groups (peptide linkage, carboxyl- and amino-groups), it was necessary to study initially the nature of radical attack on each group separately. Subsequently, two and then the three groups were studied collectively. The results obtained will be briefly presented in this order.

A. Reaction with OH Radicals

1. Amides

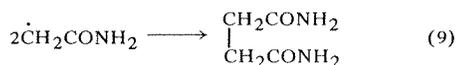
The abstraction of hydrogen atoms from acetamide by OH radicals takes place at two sites:



$$k_8 = 1.9 \times 10^8 M^{-1} \text{sec}^{-1}$$

The absorption spectra of these transients⁷ are easily distinguishable. The $\text{CH}_3\text{C}\dot{\text{O}}\text{NH}$ radicals

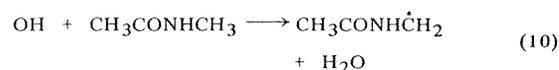
absorb below 250 nm, and the $\dot{\text{C}}\text{H}_2\text{CONH}_2$ radicals have a $\lambda_{\text{max}} = 400$ nm, $\epsilon_{400} = 1050 \text{ M}^{-1} \text{ cm}^{-1}$ (see FIGURE 1), and $\geq 60\%$ of the OH radicals react *via* eq 8. The formation of $\dot{\text{C}}\text{H}_2\text{CONH}_2$ radicals is confirmed by the high yield of succinamide⁶



with

$$2k_9 = 2.2 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}.$$

Alkylation of the amide group increases significantly the reactivity towards OH radicals and brings about a change in the site of OH radical attack. In addition, completely different transient spectra, characteristic of the intermediates produced, are observed:



$$k_{10} = 1.6 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$$

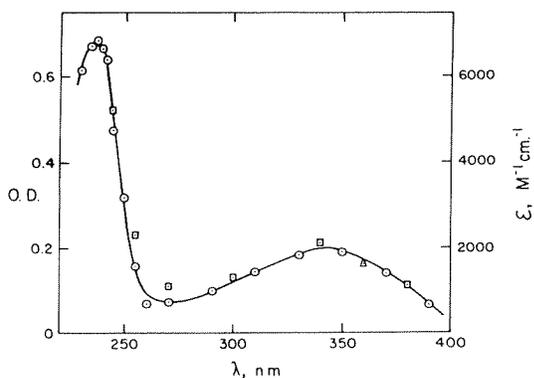
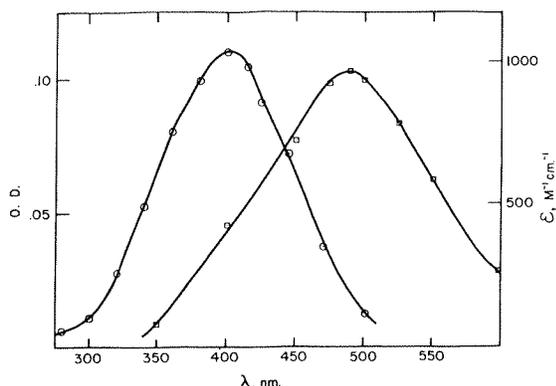


FIGURE 1

Top curve: Transient Optical Absorption Spectra of $\dot{\text{C}}\text{H}_2\text{CONH}_2$ (○) and $\text{CH}_2\text{CON}(\text{CH}_3)_2$ (□) Radicals at pH 5.5.

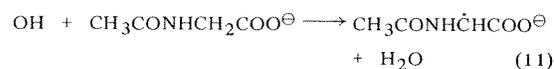
Bottom curve: Transient Optical Absorption Spectra of $\text{CH}_3\text{CONH}\dot{\text{C}}\text{H}_2$ Radicals at pH 5.0 (○) and 13.0 (□), Produced by the Reaction of OH Radicals with *N*-Methylacetamide (see Ref 7).

The $\text{CH}_3\text{CONH}\dot{\text{C}}\text{H}_2$ radicals (FIGURE 1) have maxima at 238 nm and 340 nm and extinction coefficients of 7000 and $2000 \text{ M}^{-1} \text{ cm}^{-1}$, respectively. The $\text{CH}_3\text{CONH}\dot{\text{C}}\text{H}_2$ radicals decay by second-order process with $2k = 1.3 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$. The esr spectra of $\dot{\text{C}}\text{H}_2\text{CONH}_2$ and $\text{CH}_3\text{CONH}\dot{\text{C}}\text{H}_2$ produced under steady-state radiolysis conditions have recently been observed,⁸ confirming the above results.

The work⁷ with amides establishes that abstraction from the *N*-methyl group of amides is at least one order of magnitude faster than from a α -methyl group — suggesting that amide nitrogen is much more effective in activating the methyl group to attack by an oxidizing species.

2. *N*-Acetyl Peptides

The amino group considerably affects the nature of the reactions and the reactivity of the molecule towards attack by OH radicals (and e_{aq}^- , see below), as observed in the pulse radiolysis of aliphatic amines⁹ and simple peptides¹⁰ (see below). With *N*-acetyl peptides one can, therefore, study the reactions of OH radicals with the peptide and carboxyl groups without introducing the added complexities due to the amino group. The reactivity of OH radicals with *N*-acetyl peptides is moderately high, increasing with the number of monomeric units. The main reaction for the simplest one, *i.e.*, *N*-acetyl glycine, is:

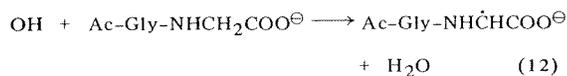


$$k_{11} = 4.2 \times 10^8 \text{ M}^{-1} \text{ sec}^{-1} \text{ (ref 10).}$$

The rate is slightly reduced (about a factor

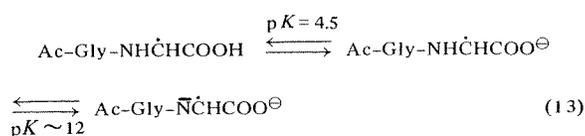
of two) when the carboxyl group of the parent compound is protonated, in agreement with the reactivity of other carboxylic acids.

With *N*-acetylglycylglycine (Ac-Gly-Gly), the main radical is probably Ac-Gly-NH \dot{C} HCOO $^-$:



$$k_{12} = 7.8 \times 10^8 \text{ M}^{-1} \text{ sec}^{-1}$$

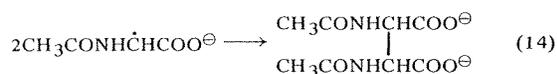
FIGURE II shows the transient spectra observed. The pH-dependence is explained as due to the acid-base equilibria of the radical:



The first pK value of the radical is about one unit higher than the pK value of the parent compound ($pK = 3.6$), indicating the effect of the unpaired electron, in an α -position to the carboxyl group, in reducing the acidity of the carboxyl group. This effect has been observed¹¹ for other substituted aliphatic acids. The spectral changes observed in alkaline solution (FIGURE II) are

attributed to the ionization of the peptide hydrogen in an α -position to the unpaired electron.

The radicals produced in reactions 12 and 13 appear to dimerize preferentially; thus, the α - α_1 -diaminosuccinic acid derivative has been measured⁶ as the main product of reaction 14:



$$k_{14} = 8 \times 10^8 \text{ M}^{-1} \text{ sec}^{-1}$$

In the presence of oxidizing agents such as H_2O_2 , Fe^{3+} , and O_2 , these radicals do not dimerize and lead instead to the formation⁶ of dehydro-derivatives, which are not stable and hydrolyze to form ammonia and keto-acids.

3. Peptides

The reactivity of peptides with terminal amino groups towards OH radicals is markedly dependent on pH, see TABLE 1. These variations in reaction rate constants can be used to determine the change in the sites of OH radical attack with change in the ionic form of the peptide molecule. The radicals produced from the

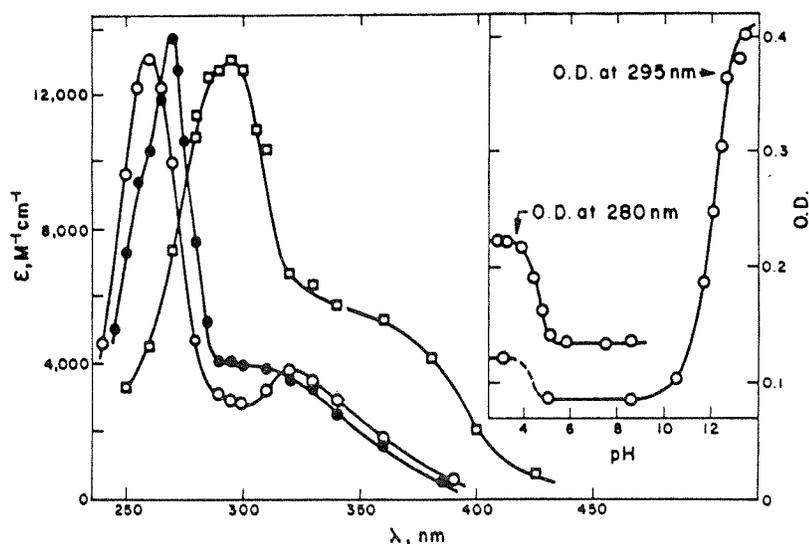


FIGURE II

Absorption Spectra of Intermediates Produced by the Reaction of OH Radicals with 10^{-2} M *N*-Acetylglycylglycine at pH 3.2, ●; pH 8.6, ○; pH 13.2, □

Insert: O. D. vs. pH Curve of Transient Monitored at 280 nm and 295 nm

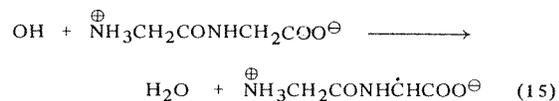
TABLE I

Rates of Reaction of OH Radicals with Simple Peptides, at Various pH Values in Aqueous Solution^a

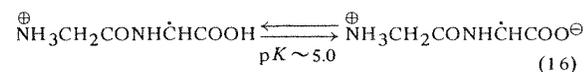
Solute	pH	Form	$k(\text{OH} + \text{S}), M^{-1}\text{sec}^{-1b}$
acetamide	5.5	CH_3CONH_2	1.9×10^8
<i>N</i> -methylacetamide	5.5	$\text{CH}_3\text{CONHCH}_3$	1.6×10^9
<i>N,N</i> -dimethylacetamide	5.5	$\text{CH}_3\text{CON}(\text{CH}_3)_2$	3.5×10^9
<i>N</i> -acetylglycine	8.7	Ac-Gly-O^-	4.2×10^8
<i>N</i> -acetylalanine	9.2	Ac-Ala-O^-	4.6×10^8
<i>N</i> -acetylglycylglycine	8.6	Ac-Gly-Gly-O^-	7.8×10^8
glycine	1.0	$\text{H}_2^+\text{-Gly-OH}$	1.6×10^7
	5.2	$\text{H}_2^+\text{-Gly-O}^-$	1.6×10^7
	10.8	H-Gly-O^-	5.0×10^9
diglycine	5.2	$\text{H}_2^+\text{-Gly-Gly-O}^-$	4.4×10^8
	10.5	H-Gly-Gly-O^-	5.2×10^9
triglycine	5.4	$\text{H}_2^+\text{-Gly-Gly-Gly-O}^-$	7.3×10^8
	10.6	H-Gly-Gly-Gly-O^-	5.0×10^9
glycine anhydride	5.0;11.0	$\text{CH}_2\text{CONHCH}_2\text{CONH}$	1.2×10^9
alanine anhydride	5.0;11.0	$\text{CH}(\text{CH}_3)\text{CONHCH}(\text{CH}_3)\text{CONH}$	1.8×10^9
sarcosine anhydride	5.0;11.0	$\text{CH}_2\text{CON}(\text{CH}_3)\text{CH}_2\text{CON}(\text{CH}_3)$	2.6×10^9

^a derived using thiocyanate method.^b data from ref 7, 10, and 12.

reaction of OH with glycylglycine absorb strongly in the uv region (FIGURE III) and are in equi-



rium with the protonated forms, as in the case of Ac-Gly-Gly:



There is an overlap between this equilibrium and the change in the site of attack by OH radicals (see below), such that the pK of reaction 16 cannot be well defined. Nevertheless, it is (FIGURE III) significantly higher than the pK of the parent compound ($pK_a = 3.06$).

The change in the site of attack is induced by the deprotonation of the terminal amino group of the peptide — this leads to increase in reactivity at this end of the peptide molecule. It is not, at present, possible to specify the exact

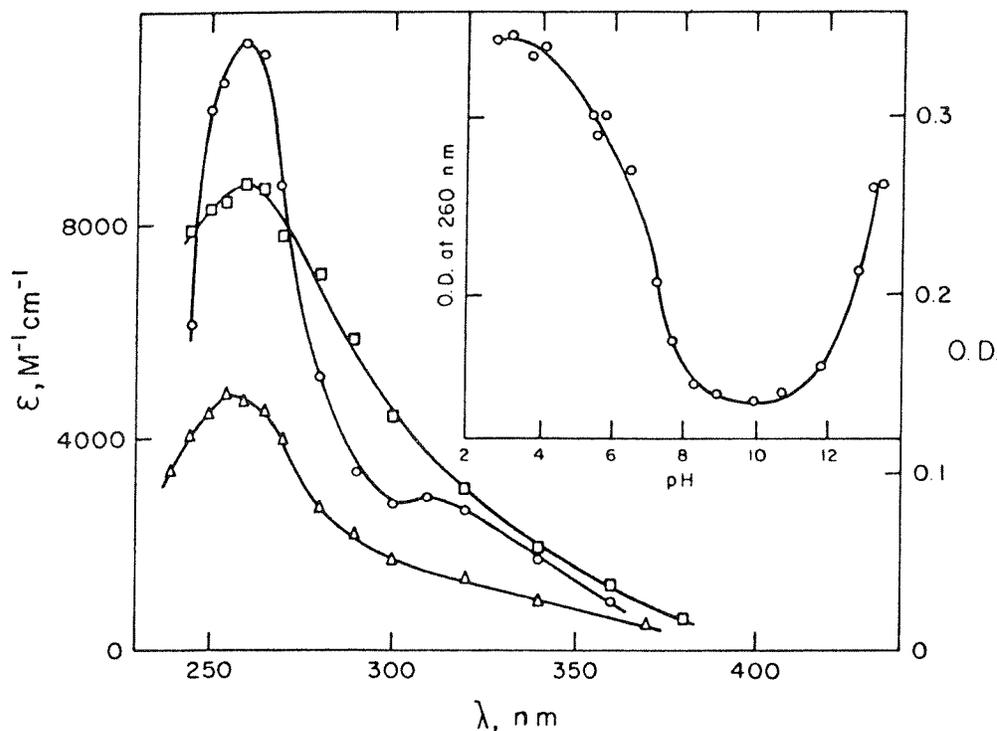
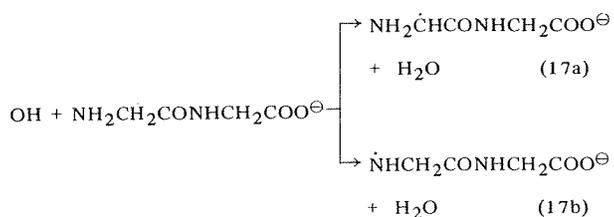


FIGURE III

Absorption Spectra of Intermediates Produced by the Reaction of OH Radicals with 0.1 M Diglycine at pH 2.8, ○; pH 10, △; pH 13.5, □

Insert: O. D.-260 vs. pH Curve of Transient

changes taking place with increase in pH (FIGURE III); two reactions are proposed and both of these may be occurring:



Similar results were observed⁹ in the reaction of OH radicals with methylamine, when H-atom abstraction from both C-H and N-H bonds occurs. The change in the spectrum above pH 11.0, FIGURE III, is believed to be due mainly to ionization of the N-H bond of the amino group.

The reaction of OH radicals with triglycine leads to similar¹⁰ results and pH effects.

All the above-mentioned radicals disappear in a radical-radical bimolecular reaction; but most of the products of these reactions have not been investigated.

4. Cyclic Peptides

The effect of high-energy radiation on the simple di- and tri-peptides mentioned above produce effects due to the terminal groups which may not be found with long peptide chains, such as found in proteins and enzymes. The simplest molecule which, in some ways, may resemble polypeptides is glycine anhydride:



In FIGURE IV are shown the spectra of

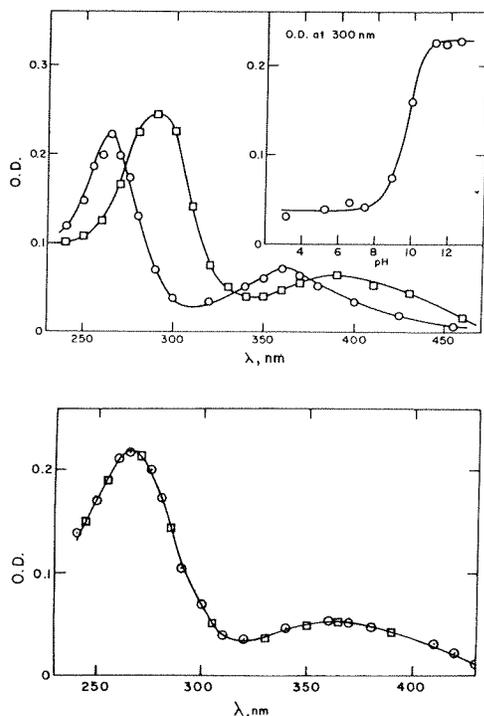
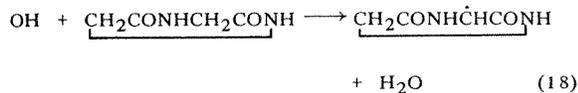


FIGURE IV

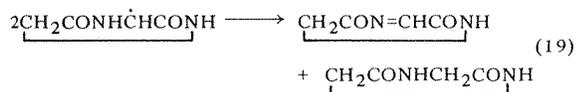
Absorption Spectra of Intermediates Produced by Reactions of OH Radicals With:

- a. top curve, 20 mM Glycine Anhydride at pH 5.0 (○) and pH 11.2 (◻)
 b. bottom curve, 4 mM Sarcosine Anhydride at pH 5.5 (○) and pH 12.4 (◻)

the transients resulting from the reaction of OH radicals with glycine anhydride:



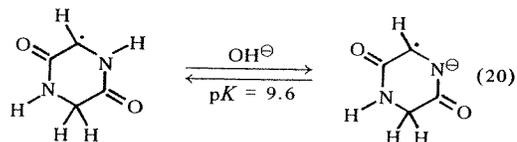
The decay of these radicals is bimolecular, and disproportionation probably results with the formation of the dehydro-derivative, as suggested by Garrison.⁶



$$k_{19} = 1.3 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$$

Some recombination of these radicals cannot be excluded.

In alkaline solutions, at $\text{pH} > 8$, the spectrum of this transient is significantly different (FIGURE IV), and a $\text{p}K(\text{radical}) = 9.6$ is obtained. Identical results and $\text{p}K$ are obtained with alanine anhydride.¹² Sarcosine anhydride, however, shows the same transient absorptions, but these remain unchanged in alkaline solution, see FIGURE IV. Based on the results with sarcosine anhydride, and on the observed¹² ionic strength effect on the decay kinetics of the transients produced in alkaline solutions of glycine and alanine anhydrides (indicating the presence of a single charge on the radicals), the acid-base properties of these radicals are attributed to the ionization of the peptide hydrogens:



Some delocalization of this charge could take place.

B. Reaction with e_{aq}^-

We have seen above that the sites of attack of hydroxyl radicals with simple peptides are mainly at the C-H bonds, and these reactions lead to H atom abstraction. When the reactions of hydrated electrons are evaluated, only the functional groups (or combination of functional groups) with an electrophilic character need be considered.

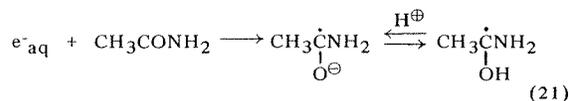
The -COOH group has an electrophilic character, while its conjugate base, -COO⁻, has very little, if any. Since at near-neutral biological pH's the carboxyl groups of most peptides are in their basic forms, their role is probably not too significant. However, the corresponding esters, -COOR, and amides, -CONH₂, do considerably affect the reactivity of the peptide molecule to attack by e_{aq}^- (see more below). The main concern here will be with the amino group and the peptide linkage.

1. Amides

All amides contain a carbonyl group, $> \text{C} = \text{O}$,

which normally has a high reactivity towards e_{aq}^- . The measured rate constants of e_{aq}^- with simple amides reveal, however, a relatively low reactivity (see TABLE II and ref 13), due probably to the mesomeric effect of the $-\text{NH}_2$ group on the carbonyl amide, which reduces the double bond character of the $> \text{C} = \text{O}$.

The reaction of e_{aq}^- with acetamide gives a transient with a low-lying absorption spectrum, $\lambda_{\text{max}} < 250 \text{ nm}$, and an $\epsilon_{250} \sim 2500 \text{ M}^{-1} \text{ cm}^{-1}$ at pH 6.0.



Other amides are expected to give similar transients. The rate of reaction of e_{aq}^- with oxamide is, however, very high (TABLE II), presumably because of the resonance stabilization of the resulting transient species. Very high reactivity of e_{aq}^- with amides with conjugated double bonds (*e.g.*, acrylamide and benzamide²) is also based on resonance stabilization.

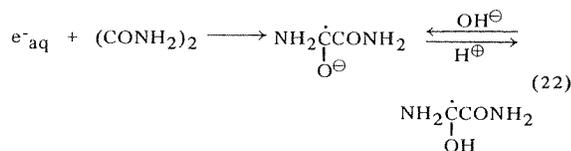
TABLE II

Rates of Reaction of e_{aq}^- with Simple Peptides in Aqueous Solution

Solute	pH	Form	$k(e_{\text{aq}}^- + \text{S}), \text{M}^{-1}\text{sec}^{-1a}$
acetamide	9.2	CH_3CONH_2	6.5×10^7
<i>N,N</i> -dimethylacetamide	9.2	$\text{CH}_3\text{CON}(\text{CH}_3)_2$	2.1×10^7
oxamide	9.2	$(\text{CONH}_2)_2$	2.7×10^{10}
<i>N</i> -acetylglycine	11.5	Ac-Gly-O^-	2.6×10^6
<i>N</i> -acetylglycylglycine	11.2	Ac-Gly-Gly-O^-	6.4×10^7
glycine anhydride	9.2	$\text{CH}_2\text{CONHCH}_2\text{CONH}$	1.7×10^9
sarcosine anhydride	9.2	$\text{CH}_2\text{CON}(\text{CH}_3)\text{CH}_2\text{CON}(\text{CH}_3)$	2.0×10^9
glycine	6.2	$\overset{+}{\text{H}}_2\text{-Gly-O}^-$	8.3×10^6
	11.8	H-Gly-O^-	1.7×10^6
diglycine	6.4	$\overset{+}{\text{H}}_2\text{-Gly-Gly-O}^-$	3.7×10^8
	13.1	H-Gly-Gly-O^-	4.9×10^7
glycine amide	6.5	$\overset{+}{\text{H}}_2\text{-Gly-NH}_2$	2.1×10^9
	11.4	H-Gly-NH_2	2.8×10^8
diglycine amide	5.7	$\overset{+}{\text{H}}_2\text{-Gly-Gly-NH}_2$	4.2×10^9
	11.4	H-Gly-Gly-NH_2	1.7×10^9
glycine methylester	5.3	$\overset{+}{\text{H}}_2\text{-Gly-OCH}_3$	6.8×10^9
	11.2	H-Gly-OCH_3	3.3×10^8

^aData from ref 2, 12-14

Transient spectra with $\lambda_{\text{max}} \sim 270$ nm and $\epsilon_{270} \sim 7500 M^{-1} \text{ cm}^{-1}$ at pH 5.0 have been observed from the reaction of e_{aq}^- with oxamide. These transients show a pH dependence indicative of the following acid-base equilibrium:



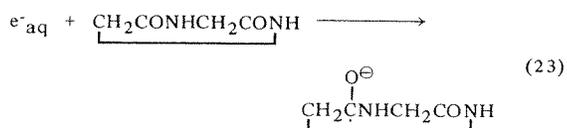
2. N-Acetyl Peptides

N-acetyl peptides containing only one peptide group, as in N-acetyl glycine, are not very reactive towards e_{aq}^- ; $k(e_{\text{aq}}^- + \text{Ac-Gly-O}^-) = 2.6 \times 10^6 M^{-1} \text{ sec}^{-1}$ and is comparable to $k(e_{\text{aq}}^- + \text{H-Gly-O}^-) = 1.7 \times 10^6 M^{-1} \text{ sec}^{-1}$. Their reactivity increases with increase in the number of peptide groups in the molecule, or with amidation or esterification of the terminal carboxyl group (see TABLE II). It would appear that the $-\text{COO}^-$ group, close to the $-\text{CONH}-$ group, reduces the cross-section efficiency of the peptide linkage for reaction with e_{aq}^- . The hydrated electrons are localized on the carbonyl-peptide group, and transient species due to $\text{R}-\overset{\cdot}{\text{C}}\text{NH}-$ have been observed by pulse radiolysis.

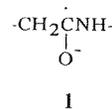
3. Cyclic Peptides

The high reactivity of the cyclic dipeptides of glycine, alanine, and sarcosine, $k(e_{\text{aq}}^- + \text{S}) \cong 2 \times 10^9 M^{-1} \text{ sec}^{-1}$, is rather surprising in view of the much lower reactivity towards e_{aq}^- of the corresponding linear peptides (TABLE II). Presumably resonance stabilization of the cyclic structure of these peptides can account for these observations.

The reaction of e_{aq}^- with glycine anhydride in neutral solution leads to the formation¹² of a short-lived intermediate with an absorption maximum below 250 nm:



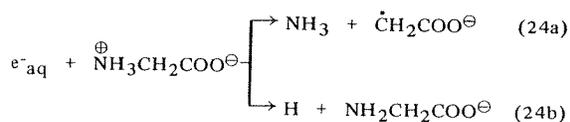
It appears that in neutral solution at pH 5.0, the electron adduct may be in the form of radical 1.



Furthermore, it was found¹² that the electron in radical 1 can be transferred to other molecules, e.g., aromatic ketones and aliphatic disulfides (RSSR) with high rate constants, $k \geq 10^9 M^{-1} \text{ sec}^{-1}$. These electron transfer reactions lead to the formation of the corresponding ketyl radicals or (RSSR) $^\cdot$ radical ions.

4. Peptides

The reaction of e_{aq}^- with the simplest amino acid, glycine, will be described first since it helps to establish the general trend encountered in oligopeptides. The reactivity of the $-\overset{\cdot}{\text{N}}\text{H}_3$ group with e_{aq}^- is rather low with aliphatic amines,² $k(e_{\text{aq}}^- + \text{CH}_3\overset{\cdot}{\text{N}}\text{H}_3) \sim 2.0 \times 10^6 M^{-1} \text{ sec}^{-1}$, and is comparable to the reactivity of the NH_4^+ ion. Introduction of a carboxyl group in an α -position to the amino group increases this rate, and the reaction leads to deamination and conversion of e_{aq}^- to H atoms:



$$k_{24} = 8.3 \times 10^6 M^{-1} \text{ sec}^{-1}$$

Deamination has been shown in the steady state⁶ radiolysis of glycine, and the formation of the $\overset{\cdot}{\text{C}}\text{H}_2\text{COO}^-$ radical in pulse radiolysis experiments.¹⁶ The $\overset{\cdot}{\text{C}}\text{H}_2\text{COO}^-$ radical has an absorption maximum at 350 nm and $\epsilon_{350} = 800 M^{-1} \text{ cm}^{-1}$. The reactivity of e_{aq}^- with glycine decreases with increase in pH (FIGURE V), and depends on the state of protonation of the amino group. The activity of β - (or γ -) amino acids, e.g., β -alanine and γ -aminobutyric acid, towards e_{aq}^- is considerably lower than that of α -amino acids, and the deamination efficiency is also reduced (see below).

The relationship proposed by Braams¹⁷ between the e_{aq}^- rate constant for the zwitterion form of the various amino acids and the pK value of the amino group was partially successful; it is clear that the presence of aromatic, -S-S-, and unsaturated residues play an important role, e.g.;

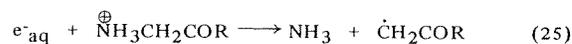
$$k(e_{aq}^- + \text{trp}) = 3 \times 10^8 \text{ M}^{-1} \text{ sec}^{-1}$$

and

$$k(e_{aq}^- + \text{cystine}) = 8.7 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1} \quad (\text{ref } 2)$$

The reaction of e_{aq}^- with oligopeptides increases with increase in the number of peptide units and is dependent on the protonation of the terminal amino group (see FIGURE V and TABLE II). Almost quantitative (> 90%) reductive deamination by e_{aq}^- was observed for oligopeptides

(from di- to hexa-glycine). This reaction was followed by observing the $\cdot\text{CH}_2\text{COR}$ radical produced in reaction 25:



The absorption spectra of the $\dot{\text{C}}\text{H}_2\text{COR}$ radicals have maxima in the 400–450 nm region and $\epsilon \sim 1000 \text{ M}^{-1} \text{ cm}^{-1}$, i.e., they are similar to the $\dot{\text{C}}\text{H}_2\text{CONH}_2$ spectrum (FIGURE I) and have similar extinction coefficients. Identical results were obtained for the other oligopeptides. Deamination seems to be the main reaction which e_{aq}^- undergo with these oligopeptides. The detailed mechanism remains to be established. Since the $\cdot\text{CH}_2\text{COR}$ radicals are observed immediately after the electron pulse, at $\tau \sim 0.1 \mu\text{sec}$, any intermediate step in the mechanism leading to deamination must be extremely fast. Work is in progress to establish the sequence of reactions leading to the deamination of long-chain peptides by solvated electrons.

The reactivity of simple peptides with terminal amide groups towards e_{aq}^- is considerably greater than that of the corresponding carboxyl group, e.g.,



$$k_{26} = 2.1 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$$

The deamination efficiency appears to be almost quantitative (> 80%). On deprotonation of the amino group, the e_{aq}^- rate drops to $2.8 \times 10^8 \text{ M}^{-1} \text{ sec}^{-1}$. Deamination of the higher homologues (e.g., $\overset{\oplus}{\text{H}}_2\text{Gly-Gly-Gly-NH}_2$) is equally efficient and leads to the formation of the corresponding $\cdot\text{CH}_2\text{COR}$ radicals.

The reactivity of simple peptides with terminal ester groups, e.g., glycine methyl ester, is similar to that of peptides with terminal amide groups, see TABLE II. It is clear that blocking the terminal carboxyl group increases considerably the reactivity of simple peptides to attack by e_{aq}^- . In alkaline solutions, at pH's when the terminal amino group is deprotonated, e_{aq}^- add to the ester group to produce characteristic spectra due to the $\text{NH}_2\text{CH}_2\overset{\ominus}{\text{C}}\text{OOCH}_3$ radical.

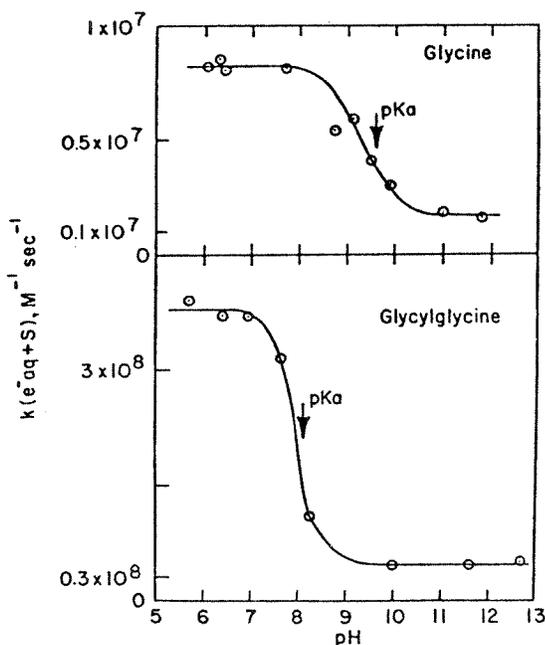
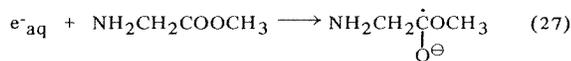


FIGURE V

Dependence Upon pH of the Rate Constant of e_{aq}^- with Glycine and Diglycine

Data for glycine (except value¹⁴ at pH 7.7) replotted from ref 15.



These radicals absorb in the uv region, with $\lambda_{\text{max}} = 265 \text{ nm}$ and $\epsilon_{265} \sim 1700 \text{ M}^{-1} \text{ cm}^{-1}$.

V. CONCLUSIONS

The reactions of hydroxyl radicals with simple (non-aromatic) peptides are dependent on the presence of terminal amino groups and on the state of protonation of the amino group. The site(s) of attack are also dependent on the location and protonation of the amino group. At pH values when the peptide is present in the form $-\overset{+}{\text{N}}\text{H}_3$, the OH radical which is electrophilic abstracts an H atom at positions furthest from the influence of the $-\overset{+}{\text{N}}\text{H}_3$ group. The resulting $-\text{CONH}\dot{\text{C}}\text{H}-$ radicals have characteristic absorption bands with maxima at $\sim 260 \text{ nm}$ and 360 nm and high extinction coefficients, up to $14,000 \text{ M}^{-1} \text{ cm}^{-1}$, with $\epsilon_{260}/\epsilon_{360} \sim 3-4$.

With long-chain peptides, or with cyclic peptides (with no terminal amino or carboxyl groups present), the role and influence of the amino group is considerably reduced. The acid-base properties of the peptide radical are expected to lead primarily to the ionization of the peptide hydrogen bond and the formation of $-\text{CON}\dot{\text{C}}\text{H}-$ radicals. These latter radicals absorb at higher

wavelengths, and also have characteristic transient spectra.

The peptide radicals normally decay by radical-radical second order reactions, leading probably to dehydro-peptide-derivatives and perhaps some dimers.

In their reaction with oligopeptides, the hydrated electron gives rise primarily to almost quantitative reductive deamination. The resulting radicals, $-\text{CH}_2\text{CONHR}$, have characteristic absorption maxima in the $400-450 \text{ nm}$ wavelength region and extinction coefficients $\epsilon \sim 1000 \text{ M}^{-1} \text{ cm}^{-1}$. The reactivity of peptides towards e_{aq}^- increases with increase in the length of the chain and hence the number of peptide linkages. It is the carbonyl-peptide group which is the first locus of the reaction of e_{aq}^- . Complete understanding of the mechanisms of the e_{aq}^- reactions with oligo- and poly-peptides, and the reactions with various residues (aromatic, $-\text{SH}$, $-\text{S-S}-$) will hopefully enable us to consider the more complex reaction mechanisms involved with proteins and enzymes. Work is actively in progress with this aim in mind.

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