Radiation Chemistry of Amino Acids and Peptides in Aqueous Solutions

M. G. Simic

Radiation chemistry relevant to radiation preservation of high protein foods is reviewed. Some conclusions concerning the chemistry of irradiated amino acids, peptides, and proteins have been derived from product analysis of γ-irradiated solutions while the main mechanistic considerations result from the chemistry and kinetics of free radical intermediates observed by pulse radiolysis. The precursors of chemistry in not too concentrated solutions (<1 M) are the water radicals e_{aq}^-, OH, and H. Their reactivity with molecules and their preference for characteristic groups within the molecule are discussed. The reviewed reactions of the model systems are accountable for a variety of radiolytic products found in irradiated foods. From detailed understanding of radiation chemistry in aqueous and frozen systems formation of many classes of compounds can be predicted or entirely eliminated in order to corroborate and extend the conclusions reached from the animal feeding experiments concerning the formation of toxic, mutagenic, and carcinogenic compounds and/or reduction of the nutritional value of foods.

Development of radiation preservation of food poses some very basic considerations regarding the possibility that ionizing radiations (γ and x rays, high-energy electrons) induce formation of toxic, mutagenic, and carcinogenic materials and/or reduce the nutritional value of foods. Whatever products are formed, their absolute yields and the relationship to the dose of radiation must be known in making a comprehensive assessment of acceptability of radiation-treated foods.

From the current knowledge of radiation chemistry of liquid and frozen aqueous solutions of meat components, it is unlikely that detrimental products in radappertized meats would be formed (in properly processed items) but more detailed investigation is desirable. While it is true that detailed radiation chemistry of foods could be very complex, yet if basic principles are known, formation of many classes of compounds could be predicted or entirely eliminated from possible mechanistic schemes with a high degree of certainty and would, without any doubt, corroborate and extend the conclusions reached from the animal feeding experiments.

In this paper we shall briefly review radiation chemistry of aqueous solutions of amino acids, peptides, and proteins with particular emphasis on pulse radiolytic data concerning free radical intermediates. So far there has been only one review of intermediates of simple aliphatic peptides (Hayon and Simic, 1971). Radiation chemistry of similar systems has been reviewed recently by Garrison (1968) and only certain aspects will be brought into discussion.

INSTRUMENTATION AND METHODS

The radiation chemical mechanisms are a result of information concerning intermediates and radiolytic products usually generated by pulse radiolysis and steady-state γ radiolysis. Classical radiation chemistry grew essentially with the development of nuclear technology. On the other hand, pulse radiolysis is of more recent origin (early 1960's). In spite of its general usefulness in physical chemistry and biochemistry, it is not widespread mainly due to its high instrumental and operational cost associated with the generators of pulsed high-energy electrons (2–10 MeV) and kinetic detection systems.

Radiation Preservation of Food Division, Food Engineering Laboratory, U.S. Army Natick Research and Development Command, Natick, Massachusetts 01760.

The techniques of pulse radiolysis have been described in detail (Hart and Anbar, 1970; Swallow, 1972). In brief, generators of pulsed electron beams such as linear accelerator, Van de Graaff, and Febetron are exploited as irradiators. On irradiation of aqueous solutions, primary free radicals e_{aq}^- (hydrated electron) and OH are formed in less than 10^{-11} s. Hence at the end of the pulse, which in most cases is 50 ns–1 μs, all the primary free radicals are formed, usually in 10^{-6}–10^{-5} M concentration region depending on the dose/pulse. These and the second generation of free radicals which result from the reaction of primary free radicals with the solute have been followed by kinetic spectrophotometry with time resolution equal to or better than the duration of the pulse. In some cases, kinetic conductivity (Asmus, 1975) and ESR (Fessenden, 1975) have been particularly useful for exact assignment of the observed transients.

PRIMARY RADIOLYTIC SPECIES

On irradiation of water at 25 °C the following species are formed (Hart and Anbar, 1970; Swallow, 1972): $H_2O \rightsquigarrow e_{aq}^-$ (2.8), H_3O^+ (2.8), OH (2.8), H (0.5), H_2 (0.4), H_2O_2 (0.8). The relative amounts, expressed here as G values (number of events/100 eV absorbed), are indicated in the parentheses. The primary free radicals e_{aq}^-, OH, and H are of transient nature and will disappear either in the reactions with each other or with radiolytic products. Most of these reactions are extremely fast on the order of 10^{10}–10^{11} M^{-1} s^{-1} (which is diffusion controlled). The same species will be formed in solutions as in pure water, provided the solute concentration is not too high (<1 M). When the electron fraction of the solute becomes comparable to that of water, a proportional amount of the absorbed dose will be lost on the direct action of the radiations on the solute with consequential formation of solute free radical.

Solute free radicals in dilute solutions (<1 M) are formed practically only in the reaction of primary water radicals with the solute. The reactivity of a solute with a particular radical is an important parameter, especially in a case where more than one solute is present. If all relevant values of reaction rate constants, k, are known, involvement of a particular solute can be easily calculated. The k values can be obtained either from the disappearance of absorbance of the reactant or from the formation of absorbance of the intermediate. Hydrated electrons have a strong absorption band in the red (Hart and Anbar, 1970), and their rates (Anbar et al., 1973) are usually determined from the decay kinetics (Tables I and II). On

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Table I. Rate Constants for Reaction of e_{aq}^- with Some Aliphatic Amino Acids, Simple Oligopeptides, and Related Derivatives in Aqueous Solutions (Simic and Hayon, 1971)

Solute, S	pH	Ionic form	$k(e_{aq}^- + S)$, M^{-1} s^{-1}	Ref
Acetate	10	Ac-O^-	$<10^6$	
Acetamide	10.9	Ac-ONH_2	1.7×10^7	
Glycine	1.0	$^+H_2$-Gly-OH	4.1×10^9	*a*
	6.4	$^+H_2$-Gly-O^-	8.2×10^6	
	11.8	H-Gly-O^-	1.7×10^6	
Glycinamide	6.5	$^+H_2$-Gly-NH_2	2.1×10^9	
	11.4	H-Gly-NH_2	2.8×10^8	
Glycinemethyl ester	5.3	$^+H_2$-Gly-OCH_3	6.8×10^9	
	11.2	H-Gly-OCH_3	3.3×10^8	
Diglycine	6.4	$^+H_2$-$(Gly)_2$-O^-	3.7×10^8	
	11.6	H-$(Gly)_2$-O^-	3.3×10^7	
Triglycine	6.1	$^+H_2$-$(Gly)_3$-O^-	1.8×10^9	
Pentaglycine	6.1	$^+H_2$-$(Gly)_5$-O^-	4.0×10^9	*b*
$(Ala)_{20}$	6.0	$^+H_2$-$(Ala)_{20}$-O^-	1.2×10^{10}	*c*
N-Acetylglycine	9.2	Ac-Gly-O^-	2.6×10^6	
N-Acetyldiglycine	9.2	Ac-$(Gly)_2$-O^-	6.4×10^7	
N-Acetyltriglycine	9.2	Ac-$(Gly)_3$-O^-	4.4×10^8	*b*

[a] Neta et al., 1972. [b] Rao and Hayon, 1974. [c] Tal and Faraggi, 1975.

Table II. Rate Constants for Reaction of e_{aq}^- with Most Reactive Amino Acids in Aqueous Solutions (Anbar et al., 1973)

Solute, S	pH	Ionic form	$k(e_{aq}^- + S)$, M^{-1} s^{-1}
Phenylalanine	6.3	$^+H_2$-Phe-O^-	1.5×10^8
	11.2	H-Phe-O^-	1.3×10^7
Tyrosine	5.8	$^+H_2$-Tyr-O^-	1.6×10^8
	11	H-Tyr-O^-	1×10^7
Tryptophan	6.8	$^+H_2$-Trp-O^-	4.0×10^8
	11.5	H-Trp-O^-	1.3×10^8
Histidine	5	$^+H_2$-His(H^+)-O^-	7×10^9
	7	$^+H_2$-His-O^-	6×10^7
	11	H-His-O^-	1×10^7
Arginine	6	$^+H_2$-Arg-O^-	1.5×10^8
	11	H-Arg-O^-	4.5×10^7
Cysteine	6.3	$^+H_2$-Cys-O^-	8.7×10^9
Cystine	6.1	$^+H_2$-Cys-O^-	1.3×10^{10}
		|	
		$^+H_2$-Cys-O^-	
	12	H-Cys-O^-	3.4×10^9
		|	
		H-Cys-O^-	

Table III. Rate Constants for Reaction of OH Radicals with Amino Acids, Simple Peptides, and Related Derivatives in Aqueous Solutions (Dorfman and Adams, 1973)

Solute, S	pH	Ionic form	$k(OH + S)$, M^{-1} s^{-1}
Acetamide	5.5	CH_3CONH_2	1.9×10^8
N-Methylacetamide	5.5	$CH_3CONHCH_3$	1.6×10^9
N-Acetylglycine	8.7	Ac-Gly-O^-	4.2×10^8
Glycine	5.2	$^+H_2$-Gly-O^-	1.6×10^7
	10.8	H-Gly-O^-	5.0×10^9
Diglycine	5.2	$^+H_2$-$(Gly)_2$-O^-	4.4×10^8
	10.5	H-$(Gly)_2$-O^-	5.2×10^9
Leucine	5.5	$^+H_2$-Leu-O^-	1.6×10^9
Phenylalanine	6	$^+H_2$-Phe-O^-	6.6×10^9
Histidine	6-7	$^+H_2$-His-O^-	5.0×10^9
Tryptophan	6.1	$^+H_2$-Trp-O^-	1.4×10^{10}

the other hand, OH radicals and H atoms have unfortunately only a weak band around 200 nm and their k values (Dorfman and Adams, 1973; Neta, 1972) are determined either from the formation kinetics of the intermediate or from competition kinetics (Table III).

In most cases the reactions of H atoms are similar to those of either e_{aq}^- or OH and, because of their relatively small G value, they will be ignored in this text.

DISCUSSION

Reactions of Hydrated Electrons with Amino Acids and Peptides. Hydrated electrons are unreactive toward hydrocarbon, hydroxy, and CO_2^- groups (Anbar et al., 1973). Hence, $CH_3CO_2^-$ has $k < 10^6$ M^{-1} s^{-1} (see Table I). On the other hand, the peptide bond, –CONH–, exhibits an affinity toward e_{aq}^- which results in the formation of electron adducts to the peptide bond (eq 1) where e^- re-

$$e_{aq}^- + CH_3\text{-}\left[\begin{array}{c}CONHCH\\ \vert\\ RCH_2\end{array}\right]_n\text{-}CO_2^- \rightarrow CH_3\text{-}\left[\begin{array}{c}CONHCH\\ \vert\\ RCH_2\end{array}\right]_n\dot{}\text{-}CO_2^- \quad (1)$$

sides most likely on one of the peptide bonds.

Increasing the number of peptide bonds greatly increases the reactivity toward hydrated electrons (Simic and Hayon, 1971), which is evident from the *N*-acetylpolyglycine series (Table I). The electron adduct is apparently protonated

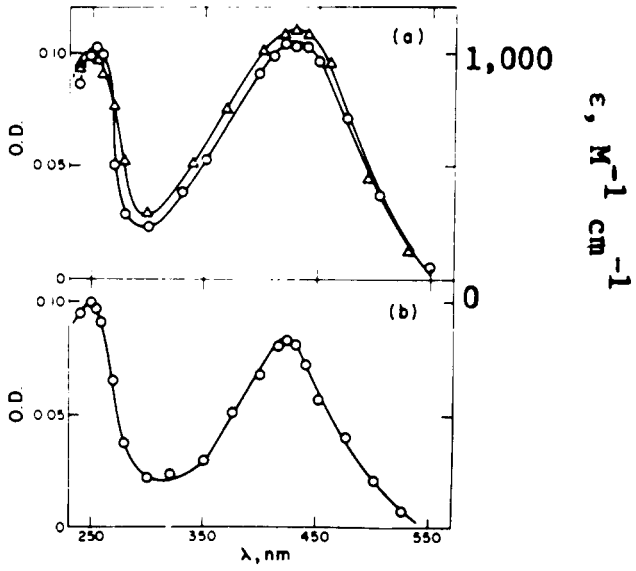


Figure 1. Transient absorption spectra produced from the reaction of e_{aq}^- with (a) 20 mM triglycine at pH 5.3, ○; and 20 mM *N*-chloracetyl glycylglycine at pH 9.2, △; (b) 5 mM tetraglycine at pH 5.3, ○. Total dose ~19 krads/pulse. Reprinted with permission from Hayon and Simic (1971). Copyright Intra-Science Research Foundation.

in neutral solutions (Rao and Hayon, 1974) since the following equilibrium has been established:

$$-\dot{C}(O^-)NH- + H^+ \rightleftharpoons -\dot{C}(OH)NH- \qquad pK_a > 12 \qquad (2)$$

Similar observations were made for proper oligo peptides (Simic and Hayon, 1971; Rao and Hayon, 1974), e.g., for $(Ala)_{20}$ the rate constant (Tal and Faraggi, 1975) approaches the diffusion-controlled limit, $k = 1.2 \times 10^{10}$ M^{-1} s^{-1}. The fate of the electron in the latter case is considerably different from reaction 1 due to the involvement of the protonated end amino group $-NH_3^+$, leading eventually to the elimination of ammonia in aliphatic oligopeptides (Garrison, 1968; Simic and Hayon, 1971; Tal and Faraggi, 1975) (eq 3). The transient spectra of the

$$e_{aq}^- + \overset{+}{N}H_3CH(R)\left[CONHCH(R)\right]_n-CO_2^- \rightarrow \cdot CH(R)\left[CONHCH(R)\right]_n-CO_2^- + NH_3 \qquad (3)$$

radicals in eq 3 are shown in Figure 1. The mechanism apparently involves formation of the electron adduct as in reaction 1 and a fast transfer of the electron by a combination of inter- and intramolecular mechanisms (Tal and Faraggi, 1975). This phenomenon is closely related to the question of conductivity of proteins in general and is relevant to mitochondrial electron transport. Further experiments are necessary for a full account of this interesting phenomenon. It may answer perhaps a question why many cytochromes have *N*-acetyl ends. The deprotonated amino group is much less electrophilic (see Figures 2 and 3), and oligopeptides with an end $-NH_2$ group behave according to reaction 1. Protonation and esterification of the carboxy group (Willix and Garrison, 1967; Simic and Hayon, 1971) greatly increase the reactivity of glycine in accordance with a general increase in the electrophilic character of $-CO_2H$ and $-CO_2R$.

All aliphatic amino acids would behave similarly to glycine although the overall charge of a molecule plays a

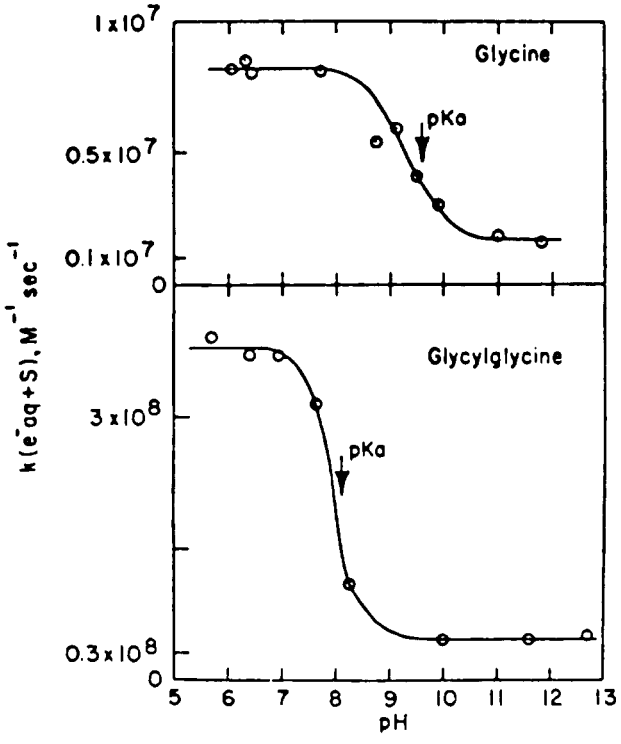


Figure 2. pH dependence of the rate constant of e_{aq}^- with glycine and diglycine. Reprinted with permission from Hayon and Simic (1971). Copyright Intra-Science Research Foundation.

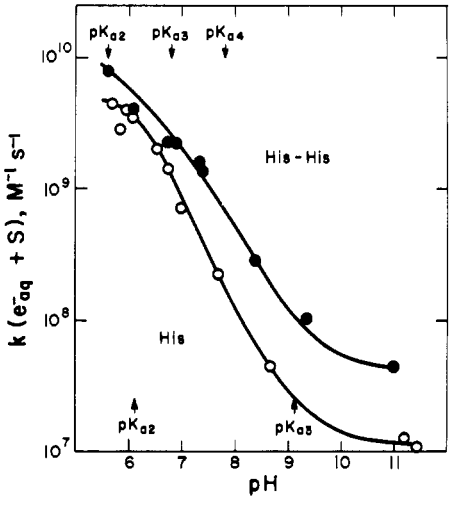


Figure 3. pH dependence of the rate constant of e_{aq}^- with histidine and histidylhistidine. p*K* values of imidazole and amino groups are indicated. Reprinted with permission from Braams (1967). Copyright *Radiation Research*.

role. For example $(Asp)_n$ has a rather low reactivity with e_{aq}^-. Amino acids with aromatic residues, e.g., Phe, Tyr, Trp, and His, are moderately reactive provided the amino group is protonated (Table II). The reactivity of His is greatly increased if the imidazole ring is protonated (pK_a = 6.0) as indicated in Figure 3. The –SH and –SS– groups are highly reactive, and their effect on the overall reactivity is evident from Table II. In general, the reactivity for the $H_2NCH(CH_2R)CO_2^-$ form closely reflects the reactivity of the residue, R, and the rate constants for the particular amino acids can be used for a rough calculation of the overall reactivity of a protein (Braams, 1967).

The fate of the electron in the reaction with a polypeptide containing electrophilic residues is not clear. The electron could attach to the end amino group (reaction 3) or to a residue, the distribution depending on the particular

Table IV. Spectral Characteristics of Transients Produced from the Reactions of e_{aq}^- and OH Radicals with Some Amino Acids and Peptides (Ph = phenyl, Im = imidazole, In = indole)

Solute, S	Free radical	pH	λ_{max}, nm	ϵ, M^{-1} cm^{-1}	Ref	Free radical	pH	λ_{max}, nm	ϵ, M^{-1} cm^{-1}	Ref
Gly-Gly	$\cdot CH_2CO$-Gly	7	435	1200	*c*	Gly-$NH\dot{C}HCO_2^-$	5	260	~8000	*i*
Phe	$HPh-CH_2CH(NH_3^+)CO_2^-$ (50%)	7	325	12000	*d*	-Ph-OH	7	310	~4000	*j*
	$Ph-CH_2\dot{C}HCO_2^-$ (50%)		<350	Low	*e*					
His	$-\dot{I}mH_2$	5.2	295	4700[a]	*f*	-Ím-OH	9.2	310	6200	*e*
			370	1700[a]				410	2300	
Trp	-ĖnH	7	310	3900[a]	*g*	-Ín-OH	7.0	320	5500	*f,k*
			390	4300[a]				360	3700	
								430	1500	
								530	1000	
Cys_2	$-(SS)^{\cdot-}$	7	410	9000	*h*	-(S̈S̈)- (50%)	7	410	2500	*b,l*
						-SS̈- (50%)				
						OH				

[a] Apparent values, % deamination not determined. [b] From RSSR derivatives. [c] Simic and Hayon, 1971. [d] Mittal and Hayon, 1974. [e] Rao et al., 1975. [f] Armstrong and Swallow, 1969. [g] Hoffman and Hayon, 1972. [h] Adams et al., 1967. [i] Simic et al., 1970. [j] Neta and Dorfman, 1968. [k] Redpath et al., 1975. [l] Bonifacic et al., 1975.

polypeptide. Electron addition to benzene, phenol, indole, and imadazole groups leads to adducts that are protonated (Hayon and Simic, 1974) at physiological pHs. The spectral characteristics of various electron adducts are given in Figure 4 and Table IV. The similarities of the electron adducts of imidazole and histidine are very noticeable, and it can be concluded from the values that e_{aq}^- predominantly adds to the protonated imidazole ring in histidine.

Electron reaction with sulfhydryl derivatives leads to formation of H_2S (Wilkening et al., 1968) and is of considerable concern in radiation technology since it produces an unpleasant odor:

$$e_{aq}^- + RSH \xrightarrow{H^+} \cdot R + H_2S \quad (4)$$

while addition of e_{aq}^- to a disulfide bridge leads to the formation of an intermediate with a characteristic absorption band near 400 nm (Adams et al., 1967):

$$e_{aq}^- + RSSR \rightarrow R\text{-}(SS)^{\cdot-}R \quad (5)$$

This intermediate has a limited lifetime and decomposes according to the following scheme:

$$R\text{-}(SS)^{\cdot-}R \overset{H^+}{\rightleftharpoons} RS\cdot + RSH \quad (6)$$

To a certain extent the electron reactivities of amino acids and peptides are predictable. For instance, a correlation between the $k(e_{aq}^- + \text{zwitterion})$ and pK of the amino group was introduced by Braams (1967). It exhibits a fairly close linear relationship for amino acids and simple peptides. The rate constants can be also correlated with the pK values of the carboxy group or the rate constants for base catalyzed ionization of the peptide hydrogen (Rao and Hayon, 1974) as well as to the charge on the carbonyl group (Q) (Tal and Faraggi, 1975). These relationships are not surprising since all of the above mentioned properties depend on the electrophilic character of the molecule.

Reactions of e_{aq}^- with Proteins. Proteins usually have very high reaction rate constants (Anbar et al., 1973) for e_{aq}^-, on the order of 10^{10}–10^{11} M^{-1} s^{-1}. As has been mentioned, this reactivity is a result of increasing the number of peptide bonds (Table I) and the presence of many electrophilic residues (Table II). These rates are substantially affected by the conformation and charge of the protein, so effects of ionic strength (Hoffman and Hayon, 1975), pH (Braams, 1967; Hoffman and Hayon, 1975), and temperature (Braams and Ebert, 1967) are observed. The temperature effect for ribonuclease is shown in Figure 5.

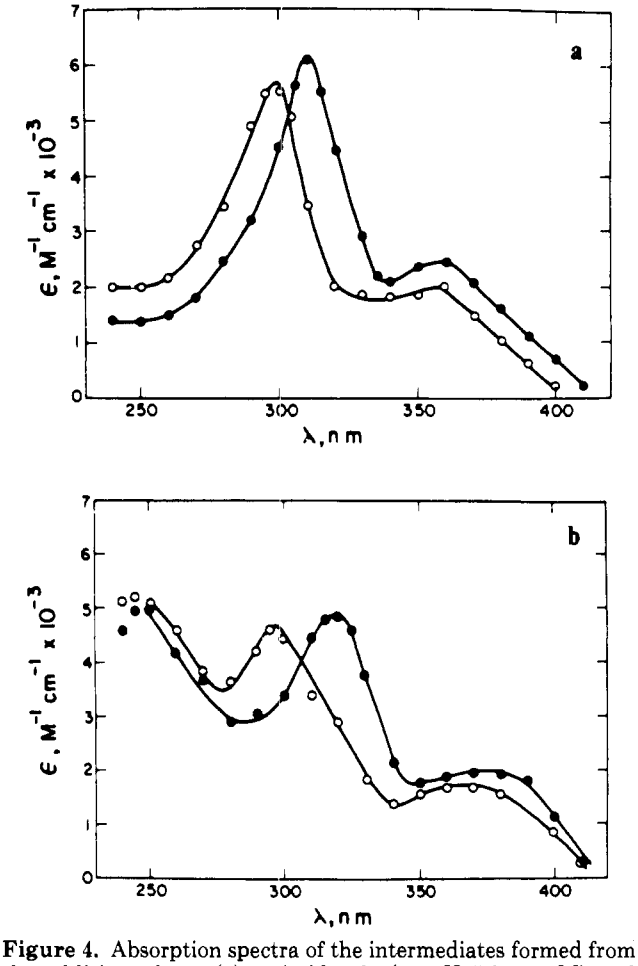


Figure 4. Absorption spectra of the intermediates formed from the addition of e_{aq}^- (a) to imidazole (○, pH 5.0, 3 mM) and *N*-methylimidazole (●, pH 5.0, 1 mM) and (b) to histidine (○, pH 5.2, 2 mM) and *N*-methylhistidine (●, pH 5.2, 2mM). A 1.0 M concentration of *t*-BuOH was present in these solutions; total dose ~2.7 krads/pulse. Reprinted with permission from Rao et al. (1975).

In reaction with redox proteins having the metal in the higher oxidation state, e_{aq}^- will predominantly end up at the metal center resulting in the reduction of the metal, e.g.

$$e_{aq}^- + cyt(Fe^{III})c \rightarrow cyt(Fe^{II})c \quad (7)$$

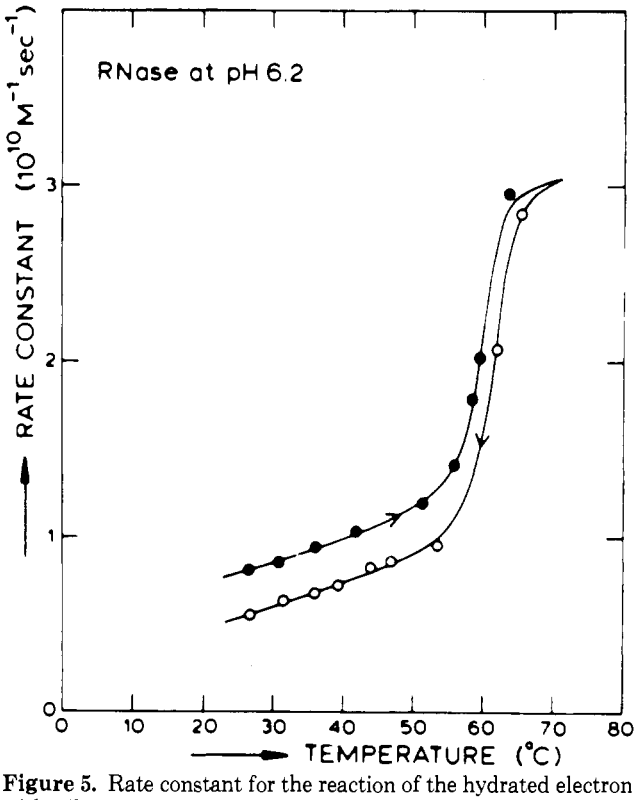


Figure 5. Rate constant for the reaction of the hydrated electron with ribonuclease as a function of temperature. Solid line, measurements taken with increasing temperature; dotted line, measurements taken on the same solution on cooling. Reprinted with permission from Braams and Ebert (1967). Copyright *International Journal of Radiation Biology*.

Consequently, cyt(III)c (Land and Swallow, 1971), Hb(III) (Clement et al., 1976), and Mb(III) (Simic and Taub, 1978) can be fully reduced by e_{aq}^- without loss of physiological functions.

In reaction with nonredox proteins, e_{aq}^- should distribute between peptide bonds and reactive residues. So far this distribution has not been demonstrated and experimental confirmation would be valuable. It is known, though, that when proteins contain –SS– groups (e.g., lysozyme), the reaction of e_{aq}^- results predominantly in $-(SS)-^{-}\cdot$ as deduced by their characteristic absorption at 420 nm.

In general, e_{aq}^- is much less efficient in inactivating enzymes (Adams et al., 1969; Adams, 1975) compared to H and OH radicals, which indicates that the predominant reaction is with peptide bonds and –SS– groups rather than with the residues of the active center (e.g., Trp).

Reactions of OH radicals with Amino Acids and Peptides. The OH radical is a strong oxidizing agent capable of oxidizing most of the naturally occurring redox systems:

$$OH + Red \rightarrow OH^- + Ox \qquad (8)$$

In the case of a metal, a higher oxidation state of the metal results, while with an organic compound the reaction leads to a cation radical, e.g., $-(SS)-^{+}\cdot$ as demonstrated by Asmus (1975).

In contrast to e_{aq}^-, the OH radical can abstract hydrogen atoms:

$$OH + RH_2 \rightarrow \cdot RH + H_2O \qquad (9)$$

The k values for reaction 9 decreases with increasng -X–H bond energies where X stands for C, N, or S. Consequently, the slowest reactions are for $-CH_3$ and $-NH_3^+$ (no reaction), while the fastest are for –SH, $-NH_2$, and $-R_2CH$ groups. Therefore, the OH rate constant for leucine is

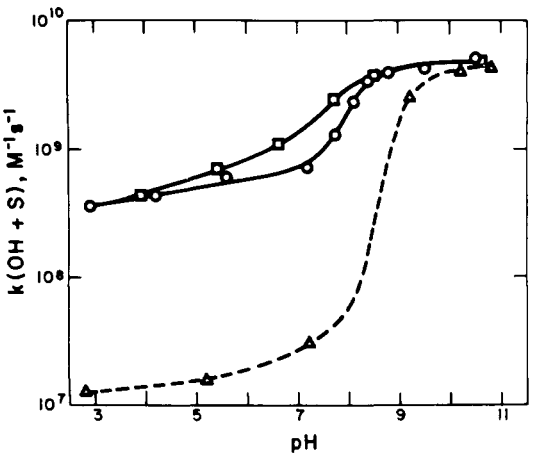


Figure 6. pH dependence of the rate constant for reaction of OH radicals with (△) glycine; (○) diglycine; and (□) triglycine. Reprinted with permission from Simic et al. (1970).

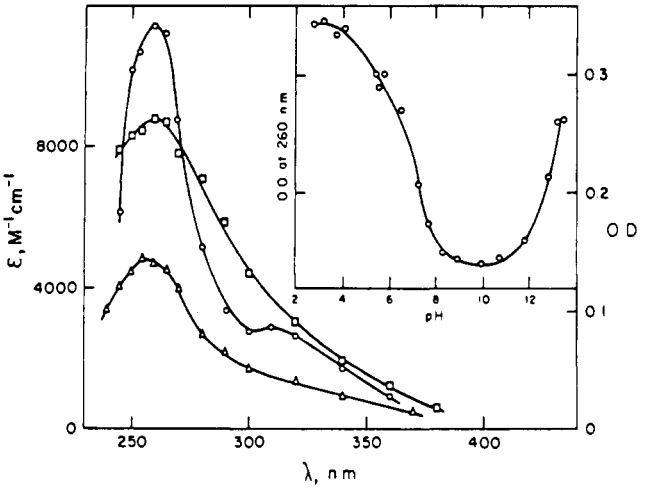


Figure 7. Absorption spectra of intermediates produced by the reaction of OH radicals with 0.1 M diglycine at pH 2.8 (○); pH 10 (△); pH 13.5 (□). Reprinted with permission from Simic et al. (1970).

higher than for glycine or alanine and is among the highest for cysteine (Table III).

On the basis of these consideration and spectroscopic evidence (Simic et al., 1970), the sites of attack of OH radicals on aliphatic peptides are predictable. For a zwitterionic form, the pattern shown in eq 10 is observed.

$$\overset{+}{NH_3}\overset{H}{C}CONH\overset{H}{C}CONH\overset{H}{C}CO_2^- \qquad (10)$$

slow: $\overset{H}{C}$ (OH) — moderately fast: $\overset{H}{C}$

For a peptide in which the amino group is not protonated, the distribution of OH attack is substantially different (eq 11). The effect of pH on k(OH + S) for glycine, diglycine,

$$NH_2\overset{H}{C}CONH\overset{H}{C}CONH\overset{H}{C}CO_2^- \qquad (11)$$

fast: $\overset{H}{C}$ (OH) — moderately fast: $\overset{H}{C}$

and triglycine is shown in Figure 6, which nicely demonstrates increase in k values on deprotonation of the amino group. The characteristic absorption spectra of the resulting diglycine radicals are shown in Figure 7.

Unsaturated compounds are extremely reactive toward OH radicals (Dorfman and Adams, 1973). The predom-

inant reaction is addition to a double bond rather than abstraction of hydrogen atoms:

$$OH + >C=C< \rightarrow >\underset{|}{\overset{OH}{\dot{C}}}C< \quad (12)$$

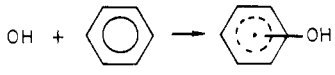
The rate constants are on the order of 10^9–10^{10} M^{-1} s^{-1}. The highest rate constants are observed for polyenes, especially the conjugated double bond systems, though unsaturated amino acids are rarely encountered (Gross, 1971).

A very important reaction of OH radicals is addition to aromatic rings:

$$OH + ArH \rightarrow HO\text{-}\dot{A}rH \quad (13)$$

where Ar stands for aromatic and heterocyclic groups. The k values for the above reaction are usually $>10^9$ M^{-1} s^{-1} but always $<10^{10}$ M^{-1} s^{-1} and are in some cases dependent on pH if the state of protonation of ArH changes.

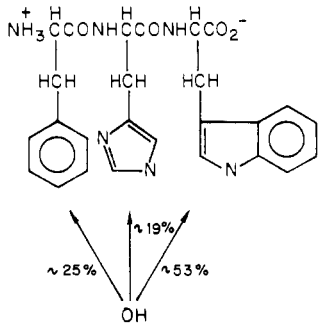
Addition of OH radical to a benzene ring and formation of a hydroxycyclohexadienyl radical was nicely demonstrated by Dorfman, et al. (1962) using pulse radiolysis (eq 14). Abstraction of H atoms and formation of the $\cdot C_6H_5$



(14)

radical is negligible in comparison to the addition. Even in aromatic compounds with aliphatic side chain, e.g., in Phe, Tyr, Trp, and His, the main reaction still will be addition to the ring. The mechanism of addition is not completely clear. It appears that initial interaction of OH radical is with the electron π system. Hence the substituent which affects the electron density of the π system affects the rates too. For example, protonation of the imidazole residue in histidine greatly decreases the rate for most benzene derivatives, $k > 10^9$ M^{-1} s^{-1}, and is less affected by the substituents. The radiolytic products exhibit a distribution of *o*-, *m*-, and *p*-hydroxy derivatives which may vary from system to system.

Based on the above considerations for k(OH + S) values, the OH reaction sites in peptides will be distributed between the peptide backbone and the residues. For instance, at pH 7 Phe-His-Trp peptide exhibits the pattern shown in eq 15 with only ~3% of OH radicals attacking



(15)

the peptide backbone. For a peptide with a proportionally fewer aromatic residues as usually encountered, this fraction becomes greater.

Spectral characteristics of transient intermediates that result from the reaction of OH radicals with several amino acids and peptides are given in Table IV. Similarity between the transient spectra resulting from the reaction of OH radicals with histidine and imidazole is demonstrated in Figure 8. Most of the kinetic and mechanistic considerations stem from the use of these data. Applications of these data to the radiation chemistry of proteins is more difficult since in many instances absorption spectra of the intermediates overlap in the narrow spectral region

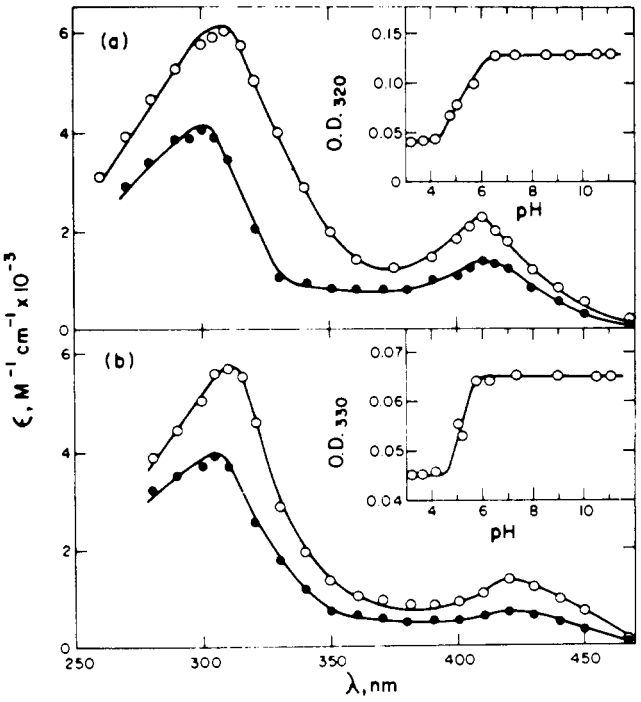


Figure 8. Absorption spectra of the intermediates formed from the reaction of OH radicals with (a) 1 mM histidine at pH 3.5 (●) and pH 9.2 (○) and (b) 1 mM *N*-methylhistidine at pH 3.8 (●) and pH 7.3 (○). Solutions were saturated with N_2O; total dose, 2.4 krads/pulse. Reprinted with permission from Rao et al. (1975).

between 300 and 350 nm and deductions from model systems become essential.

Reaction of OH Radicals with Proteins. In view of the limited number of detailed studies that have been conducted, only a few tentative generalizations can be made about OH–protein reactions. The fraction of highly reactive sites due to the aromatic and heterocyclic amino acids in proteins is much smaller than in our Phe-His-Trp example discussed above. Hence, more that 3% of OH radicals should be involved in C–H bond abstractions. Approximate calculations of the overall reactivity of a protein can be applied (Braams, 1967) using rate constants for constituent amino acids. The distribution of attack, however, would depend on the conformation of the protein. Deeply buried groups are expected to be less accessible to OH radicals and a proper distribution of the sites of attack cannot be calculated from relative k values alone. The experimentally determined k values for OH radical reactions (Dorfman and Adams, 1973) with, e.g., alcohol dehydrogenase, catalase, lyzozyme ribonuclease, trypsin, trypsinogen, etc., are very high of the order of 10^{11} M^{-1} s^{-1}, mainly due to their large size, which increases the encounter frequency.

It is interesting to note that OH radicals, in spite of their strongly oxidizing character, lead to a reduction of the metal center (Simic and Taub, 1978) as in the case of cyt(III)c. This somewhat surprising phenomenon can be explained on the premise that most of the OH radicals will react with the outer protein coat surrounding the metal ion, and only a small fraction of OH radicals can penetrate it to attack the metal directly. Some of these radicals on the protein coat can apparently transfer an electron intramolecularly to the metal center, resulting in oxidation of the protein radical and reduction of Fe(III) to Fe(II). In cyt c only 50% of the OH radicals participate indirectly in reduction, which takes place in two distinct steps with $k_1 = 2 \times 10^5$ s^{-1} and $k_2 = 3 \times 10^4$ s^{-1}. It appears that the free radicals on the protein can be subdivided into three

Table V. Initial Relative Decay Rates of $\cdot CO_2^-$ and $\cdot CH_2COO^-$ Radicals in the Presence and Absence of Various Organic Radicals (Simic and Neta, 1970) ($[\cdot CO_2^-] = [\cdot CH_2CO_2^-] \simeq [\cdot R]$), Reaction Products RCO_2^- and $RCH_2CO_2^-$, Respectively.

Organic radical, ·R	Relative $k(\cdot R + \cdot CO_2^-)$[a]	Relative $k(\cdot R + \cdot CH_2CO_2^-)$[b]
$\cdot CO_2^-$	1.0[c]	4.2
$\cdot CH_2CO_2^-$	4.5	1.0[d]
$\cdot CH_2OH$	4.9	4.0
$CH_3\dot{C}HOH$	3.9	3.8
$\cdot CH_2C(CH_3)_2OH$	3.0	2.4

[a] Decay monitored at 225 nm in solutions at pH 5. [b] Decay monitored at 350 nm in solutions at pH 9. [c] Taken as unity, absolute $k = 8.0 \times 10^8$ M^{-1} s^{-1} (Neta et al., 1969). [d] Taken as unity, absolute $k = 5.0 \times 10^8$ M^{-1} s^{-1} (Neta et al., 1969).

classes: (a) oxidizable free radicals strongly coupled to the metal, (b) oxidizable free radicals weakly coupled to the metal, and (c) nonoxidizable free radicals either insulated and/or noninteracting with the metal center.

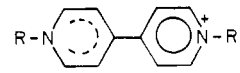
The hydroxyl radical is very efficient in inactivating many enzymes. For instance, D_{37} (dose at which 37% of activity remains) is 3.2 Krad for lysozyme (Adams et al., 1969) and 4.4 krad for α-chymotrypsin (Adams, 1975) in aqueous solutions at room temperature. This relatively high efficiency of enzyme inactivation can be attributed to the high proportion of OH radicals reacting directly with the active center, which often involves the Trp residue. As Table III shows, this particular residue is more reactive than any other in the enzyme. In the dry state, enzymes are considerably less sensitive since there would be few if any OH radicals produced and because of extensive recombination reactions. For instance, D_{37} is 26 Mrad for dry lysozyme and 17 Mrad for dry chymotrypsin, as found by Tolbert and coworkers (Okada, 1970). Similarly, due to a decrease of OH radical yield in frozen systems, the stability of enzymes is greatly increased. In fact, even a dose of 4 Mrad used for sterilization of meats is not sufficient to inactivate proteolytic enzymes in meat. [Hence, a 60–72 °C heat treatment prior to irradiation is necessary (Losty et al., 1973) for enzyme inactivation.]

Reactions of Secondary Free Radicals. *Radical–Radical Reactions.* Free radicals with few exceptions are highly unstable as expected for an entity with an unpaired electron (Griller and Ingold, 1976). The major pathway for reaction and the rate at which they react depend on the conditions such as phase, temperature, viscosity, and composition of the medium. In aqueous media at room temperature, the most common reactions for carbon radicals are shown in eq 16, with k not exceeding 10^{10} M^{-1} s^{-1} for the fastest radical–radical reactions (Hayon and Simic, 1970). Usually, $k \sim 10^9$ M^{-1} s^{-1} is observed for small electrically neutral organic free radicals. Cross reactions between unlike free radicals are also possible, e.g., in carboxylation reactions (Scholes et al., 1960) (reaction 18). Some of the carboxylation k values are given in Table V (Simic and Neta, 1970). The charge on the free radicals affects their interaction rate constants as predicted by the Debye equation (1942). The decrease in k values is not so pronounced for reaction between singly charged radicals (a factor of 2) as it is for radicals with larger charge, e.g., $k = 3 \times 10^5$ M^{-1} s^{-1} for tricarballylic acid radical (Simic et al., 1969) which has a 3– charge. Higher viscosity can also reduce the rate constants substantially. The encounter frequency of radicals is reduced in viscous media as described by the Stokes–Einstein–Smoluchowski equation (Moelwyn Hughes, 1971). In frozen systems at 77 K the radicals can last almost indefinitely since they are immobilized by the matrix (Kevan, 1969), yet they become mobile again at higher temperatures and disappear with rates dependent on the character of the matrix and the temperature (Taub, 1975).

$$\cdot RH + \cdot RH \rightarrow HR\text{-}RH \text{ (dimerization)} \quad (16a)$$

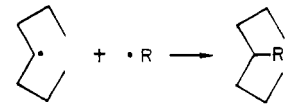
$$\cdot RH + \cdot RH \rightarrow RH_2 + R \text{ (disproportionation)} \quad (16b)$$

Whether free radicals dimerize or diproportionate depends on their oxidation and reduction potentials, some insight into which can be obtained from experiments involving fast polarographic instrumentation coupled to pulse radiolysis as introduced by Henglein (1976). For example, free radicals from reactions 3, 4, 10, and 12 would mainly dimerize while OH radical adducts of benzene derivatives (reactions 14 and 15) would substantially disproportionate (Bansal and Henglein, 1974). Viologen free radicals



would not undergo either of these reactions readily because of steric and redox considerations and are hence relatively stable (persistent) (Griller and Ingold, 1976) in the absence of O_2 and other redox solutes.

An interesting situation arises when a free radical site is produced on a very large molecule, e.g., protein or DNA. Because of steric hindrances, it might be long-lived ($t_{0.5}$ of a few seconds or minutes) or even stable as in the case of the Shethna flavoprotein semiquinone form (Edmondson and Tollin, 1971). Therefore, if a relatively immobile or "frozen" free radical is induced in a protein, it might not be able to react with another protein radical and would have to await for an encounter with a smaller mobile free radical (eq 17). This combination reaction



"frozen" mobile (17)

is easy to follow by using labeled mobile radicals and following the radioactivity of the modified protein. Carboxylation reactions, reactions of carboxyl radicals with carbon radicals, are fast reactions and illustrate the approach. Labeled carbon dioxide or bicarbonate are used as a starting material and the radioactive carboxyl groups become attached (Scholes et al., 1962):

$$\cdot R + {}^{14}\dot{C}O_2^- \rightarrow R\text{-}{}^{14}CO_2^- \quad (18)$$

thereby imparting radioactivity to the protein fractions.

In a similar fashion when free radicals are produced on lipids, proteins, and DNA, carboxylation of these molecules is observed (Scholes et al., 1963). Other, more complex, mobile free radicals can react with protein radicals. If a dilute solution of albumen in the presence of amino acids is irradiated, Phe, Trp, and His free radicals become attached to the protein (Yamamoto, 1973). Detailed mechanism for these interesting reactions has not been worked out and further studies are desirable both from the mechanistic and kinetic point of view. Since in meats about 2% weight is free amino acids, some addition of amino acid radicals to the protein is expected if irradiation would take place at room temperature.

Radical–Solute Reactions. In the previous section, radical–radical reactions of secondary free radicals were described. These are the main reactions of secondary free

radicals that will take place either under pulse (high intensity electron source) or continuous irradiation conditions (γ rays). Secondary radicals can react with a solute as well if high concentrations of solutes with weak X–H bonds are present. For example, another generation of organic free radicals is produced on H-atom abstraction by free radicals to form molecules with stronger C–H bonds (eq 19). These reactions are rather slow and are less likely

$$\cdot R + X\text{-}H \rightarrow R\text{-}H + \cdot X \qquad (19)$$

secondary radical — weak — strong — tertiary radical

in pulse radiolytic experiments where normally high initial concentrations of free radicals are produced (10^{-6}–10^{-4} M). On the other hand, in γ radiolysis where the steady-state concentration of free radicals is much lower (e.g., $\sim 10^{-10}$ M) reaction 19 has to be considered. For instance, the following reactions, (eq 20 and 21) were suggested by Garrison et al. (1967):

$$RCONHCH(CH_3)R \rightarrow RCO\dot{N}H + \cdot CH(CH_3)R \qquad (20)$$

$$RCO\dot{N}H + RCONHCH(CH_3)R \rightarrow RCONH_2 + RCONH\dot{C}(CH_3)R \qquad (21)$$

Reaction 21 is greatly enhanced in the presence of S–H bonds which are much weaker than the C–H bonds:

$$\cdot R' + RS\text{-}H \rightarrow R'H + RS\cdot \qquad (22)$$

This type of reaction has been known for a long time in radiation biology and has been studied also by pulse radiolysis. Reaction 22 is a basis of certain types of radiation protection, i.e., repair, mechanisms (Adams, 1970). One should point out that RSH compounds can also repair cation radicals by a somewhat different mechanism (electron transfer) (Willson et al., 1974). In irradiated meats reaction 22 should be considered. If the resulting RS· radicals are mobile, the following reaction takes place (Adams et al., 1967):

$$RS\cdot + RS\cdot \rightarrow RSSR \qquad (23)$$

Reaction of RS· radicals with carbon centered free radicals are less likely, though possible.

Oxidation of Free Radicals. Standard redox potentials of e_{aq}^- and OH are −2.8 and about +2 V, respectively. Therefore, free radicals produced from the reactions of these two primary species can have redox potentials in the −2.8 to +2 V region, the exact values depending on the nature of the radical produced. For instance, electron adducts to *N*-acetyl derivatives of peptides are easily oxidized with even mild oxidants, Ox, such as lipoate (Faraggi et al., 1975) or with oxygen (eq 24 and 25).

$$\cdot P^- \text{ (or } \cdot PH) + Ox \rightarrow P + Ox^- \qquad (24)$$

$$\cdot P^- + O_2 \rightarrow P + \cdot O_2^- \qquad (25)$$

The oxidation rate constants of low redox potential free radicals are usually high, $k \sim 10^9$ M^{-1} s^{-1}. Low redox potential radicals (Lilie et al., 1971) can be also formed by OH radical abstraction from C–H bonds, provided the abstraction takes place in the position α to certain functional groups. Radicals derived from hydrocarbons and free radicals having unpaired electrons localized further away from functional groups (β, γ, etc.) are much more resistant to oxidation. A generalization regarding oxidizability of aliphatic radicals is summarized in Scheme I. For radicals formed by the addition of OH to aromatic and heterocyclic residues, the generalization shown in Scheme II applies. The ·Trp–OH obviously consists of more than one kind of radicals, some of which are easily oxidizable. In most cases, the products of Scheme II have

Scheme I

$$\cdot RH + Ox \rightarrow R + Ox^- + H^+$$

Easy to oxidize	Hard to oxidize
$>\dot{C}OH$	$>CH\dot{C}HCH_3$
$>\dot{C}O^-$	$>CH\dot{C}HCH_2OH$
$>\dot{C}NH_2$	$-CONH\dot{C}H-$
$-\dot{C}(OH)NH-$	$H_3\overset{+}{N}CHCO_2^-$
	$\cdot CHR$

Scheme II

$$HO\text{-}\dot{A}rH + Ox \rightarrow HO\text{-}Ar + Ox^- + H^+$$

Easy to oxidize	Hard to oxidize
·His–OH	·Phe–OH
	·Tyr–OH

20% ·Trp–OH 80%

not been examined. An exception is Phe, where on γ radiolysis, *m*- and *p*-Tyr are observed on quantitative oxidation of HO–Phe radicals by ferricyanide (Taub et al., 1978) which parallels the oxidation of OH adduct to benzoic acid (Volkert and Schulte-Frohlinde, 1968).

Reactions with O_2. Atmospheric oxygen is in a triplet state and acts as a diradical (Kearns, 1971). Accordingly, it has a high reactivity toward a variety of free radicals. In many reactions O_2 adds and gives peroxy radicals, yet O_2 can also act directly as an oxidant (Simic, 1975), i.e., electron acceptor. These two possibilities are shown in eq 26. Reaction 26 is normally fast with $k \sim 10^9$ M^{-1} s^{-1}. The

$$\cdot RH + O_2 \rightarrow \cdot OORH \qquad (26a)$$

$$\cdot RH + O_2 \rightarrow R + \cdot O_2^- + H^+ \qquad (26b)$$

electron transfer may go through initial formation of a peroxy radical, e.g.

$$\cdot OORH \rightleftharpoons H^+ + \cdot OOR^- \rightarrow R + \cdot O_2^- \qquad (27)$$

Reaction 27 takes place usually for α-hydroxy derivatives at pH 7. More stable peroxy radicals react with each other ($k \sim 10^7$ M^{-1} s^{-1}) to give eventually the final oxidized products, e.g.

$$2\cdot OORH \rightarrow \text{tetroxide} \rightarrow R{=}O + RHOH + O_2 \qquad (28)$$

(alkoxy radicals)

The exact mechanism has not been fully resolved and may depend on the nature of a particular peroxy radical.

The superoxide radical, $\cdot O_2^-$, which is formed in a reaction of electrons with oxygen and in reaction 27 is involved in two important reactions. In the absence of strong oxidants and any other reactants, hydrogen peroxide is formed via

$$2\cdot O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \qquad (29)$$

In the presence of peroxy radicals in neutral and alkaline pHs, a competetive reaction leads to formation of hydroperoxides (Scholes and Weiss, 1959):

$$\cdot OORH + \cdot O_2^- + H^+ \rightarrow HOORH + O_2 \qquad (30)$$

with $k_{30} \sim 10^7$ M^{-1} s^{-1} (Simic and Hayon, 1974).

Implications for Irradiated High Protein Foods. The reactions of electrons (hydrated, solvated, trapped, and otherwise) with proteins lead to deamination, scission of peptide and disulfide bonds, and addition to aromatic and heterocyclic amino acid residues. The extent and ratio of these reactions depend on conditions and the protein in the system. These reactions are accountable for a

variety of radiolytic products found in irradiated foods (Taub et al., 1976).

The reactions of OH radicals with proteins lead to hydrogen atom abstraction, addition to aromatic, heterocyclic, and sulfur-containing amino acid residues as well as to oxidation of some of those residues. In frozen systems the OH radical contribution will be greatly diminished and only a small fraction of OH radical induced products will be present in radappertized meats. Nevertheless, recognition of those products is desirable.

Radiolytic products usually increase in concentration linearly with the radiation dose over a wide dose range. Some products may have a high reactivity toward radicals and could consequently reach a steady-state concentration. It is unlikely that in irradiated meats radical-product reactions would occur because of the relatively high reactivity and concentration of the protein fraction. Hence, deviation from linearity might occur only at very high doses (greater than 5 to 10 Mrads) for the most reactive radiolytic products.

Reactions of oxygen with free radicals are very fast and extensive. In the irradiation of foods packed in containers that are evacuated by commercial standards, the small amounts of remaining oxygen would be consumed at relatively low doses (under the conditions in this laboratory, at about 50 Krads) and are of lesser importance. The small amounts of peroxy compounds formed in these reactions are expected to diminish in the protein component on further irradiation.

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