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REACTION MECHANISMS IN THE RADIOLYSIS OF
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W.M. Garrison

July 1986

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LBL-18613
Revised

Reaction Mechanisms in the Radiolysis of
Peptides, Polypeptides and Proteins¹

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July 1986

¹This work was supported by the U. S. Department of Energy under
Contract No. DE-AC03-76SF00098.

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Reaction Mechanisms in the Radiolysis of
Peptides, Polypeptides and Proteins¹

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Abstract

The objective of this review is to bring together and to correlate the wide variety of experimental studies that provide information on the reaction products and reaction mechanisms involved in the radiolysis of peptides, polypeptides and proteins (including chromosomal proteins) in both aqueous and solid-state systems. The comparative radiation chemistry of these systems is developed in terms of specific reactions of the peptide main-chain and the aliphatic, aromatic-unsaturated and sulfur-containing side-chains. Information obtained with the various experimental techniques of product analysis, competition kinetics, spin-trapping, pulse radiolysis and ESR spectroscopy is included.

I. Introduction

A detailed understanding of reaction products and reaction mechanisms in the radiolysis of peptides, polypeptides and proteins is becoming of increasing importance in the development of our understanding of the precise chemical basis of radiation damage in biological and biochemical systems.

The major interest in radiation biology at the molecular level over the years has been on the nucleic acid component of the chromosome because of its primary genetic importance. A comprehensive literature on the radiation chemistry of DNA and constituent compounds has been developed; there have been a number of symposia and review articles on this subject over the past few years.¹⁻⁴ However, the more recent studies of the basic mechanisms of gene control make it increasingly evident that the conjugated chromosomal proteins also play key roles in determining the conformation and genetic activity of the nucleoprotein complex.⁵⁻⁷

Our present knowledge of the chemical actions of ionizing radiations on chromosomal proteins is relatively limited. Protein damage in a few nucleoprotein systems has been reported. The radiation inactivation of phage and other viruses in aqueous solution appears to arise in large part from damage to capsid protein caused by attack of radical species derived from water.⁸⁻¹¹ It has also been shown that much of the radiation damage to thymus nucleoprotein (deoxyribonucleohistone) in oxygenated solution arises from the destruction of histone amino acids through OH radical attack;^{12,13} this

results in a marked decrease in the stability of the DNA-histone complex.^{13,14} The direct action of ionizing radiation on solid nucleoprotein appears to involve the formation of free radical species derived from both the DNA and the protein component.¹⁵ Other studies have established that co-valent DNA-protein crosslinks are formed when whole cells or mixtures of DNA and cell proteins (histones, etc.) are exposed to uv and γ radiation.¹⁶⁻¹⁹ The core histones (H2A, H2B, H3, H4) have been identified as the chromosomal proteins predominantly involved in the formation of DNA-protein crosslinks in γ -irradiated chromatin.^{18,19} Radiation-induced crosslinking of histone octamer complexes has recently been reported;²⁰ these complexes consist of two sets of core histones and are major components of the nucleosome, a basic sub-unit of chromatin.^{21,22}

It is now well established that certain processes of aerobic cell metabolism involve the enzymatic production of the hydroperoxyl (superoxide) radical O_2^- and its subsequent removal by superoxide dismutase to yield hydrogen peroxide: $2O_2^- + 2H_2O \rightarrow H_2O_2 + 2OH^- + O_2$.^{23,24} Further, there is considerable evidence that part of the hydrogen peroxide so formed is converted to OH radicals in vivo through an iron catalyzed Haber-Weiss mechanism: $O_2^- + H_2O_2 \rightarrow O_2 + OH + OH^-$.²⁵ Hence, studies of the reactions of radiation-generated O_2^- and OH with biochemical solutes, including peptides and proteins, can have useful application in studies of the physiological chemistry of unirradiated biological systems.

An important source of information on the question of whether or not toxic or other deleterious substances are formed in the radiation sterilization of foods is the chemical study of reaction products and reaction mechanisms in the radiolysis of individual food components. Several years ago, an evaluation was made of the possible formation of deleterious products in the radiolysis of peptide derivatives of the α -amino acids common to food proteins under oxygen-free conditions.²⁶ From this evaluation of information available at that time: (1) It was concluded that formation of higher molecular-weight dimer products through intermolecular crosslinking is a characteristic feature of the radiolysis of most α -amino acids in the peptide form - both in aqueous solution and in the solid state. (2) It was pointed out that these amino acid dimer-products are of a class of compounds, the α, α' -diaminodicarboxylic acids, which are not normally present in food proteins but which do occur naturally in glycoprotein (peptidoglycan) of the bacterial cell wall. (3) On the basis of these considerations it was proposed that a program be initiated to establish whether or not the radiation synthesis of α, α' -diaminodicarboxylic acids is an important factor in determining the wholesomeness of irradiated high-protein foods. Numerous papers on the chemistry of formation of such α, α' -dimers in the radiolysis of peptides and proteins have since appeared and are included in this review.

The objective of this review is to bring together and to correlate the wide variety of experimental studies that provide information on the reaction products and reaction mechanisms involved in the

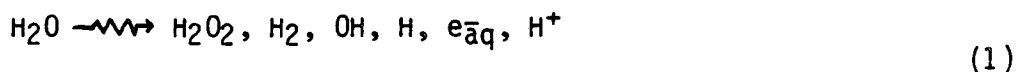
radiolysis of peptides, polypeptides and proteins (including chromosomal proteins) in both aqueous and solid-state systems. The comparative radiation chemistry of these systems is developed in terms of specific reactions of the peptide main-chain and the aliphatic, aromatic-unsaturated and sulfur-containing side-chains. Information obtained with the various experimental techniques of product analysis, competition kinetics, spin-trapping, pulse radiolysis and ESR spectroscopy is included.

II. Main-chain Chemistry of Peptides in Aqueous Solution

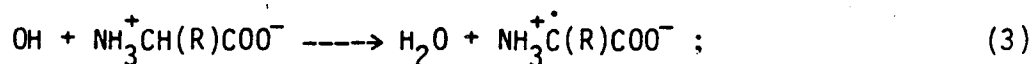
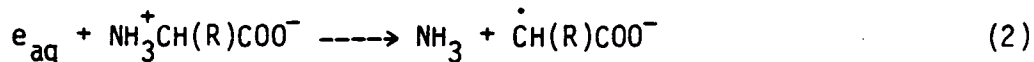
A. Background Chemistry

Studies of the chemical actions of ionizing radiations on the peptide main-chain have evolved from earlier studies of the radiation chemistry of the simpler α -amino acids as monomers both in aqueous and solid state systems.

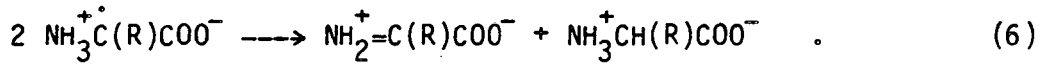
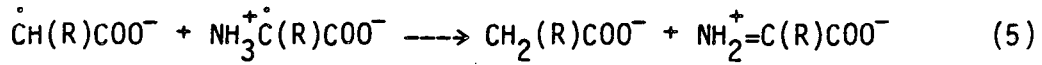
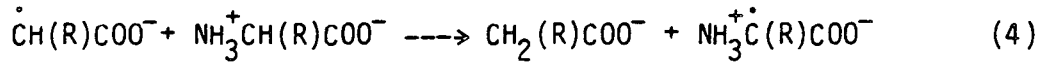
Chemical change in dilute aqueous solution is initiated by the radiation induced decomposition of water^{27,29}



where e_{aq}^- represents the hydrated electron.³⁰ For γ rays and fast electrons the yields of the radical products correspond to $G(\text{OH}) = 2.8$, $G(e_{\text{aq}}^-) = 2.7$, $G(\text{H}) = 0.55$ where $G(X)$ represents the number of species X formed per 100 eV absorbed energy. The reactions of the major radical products e_{aq}^- and OH with the amino acids glycine and alanine in oxygen free solution yield ammonia, keto acid and fatty acid as major products. Detailed chemical studies of these systems including the use of second solutes for the preferential scavenging of e_{aq}^- and OH led to identification of the principal reaction modes^{31,32}



subsequent reactions include



A small fraction of the $\text{NH}_3^+\dot{\text{C}}(\text{R})\text{COO}^-$ radicals undergo dimerization to yield α, α' -diaminosuccinic acid. The labile imino acid derivative produced in the disproportionation steps 5,6 hydrolyzes spontaneously

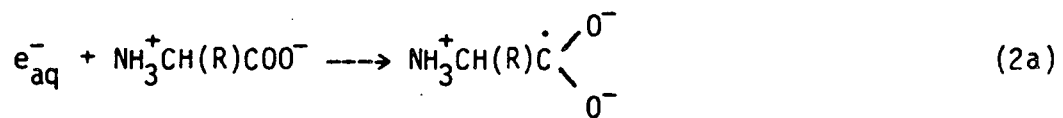


The overall stoichiometry of reactions 2-7 gives

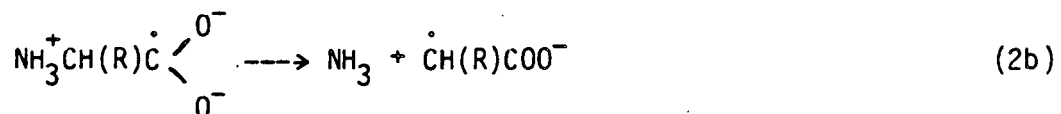
$$G(\text{NH}_3) = G(\text{RCOCOOH}) + G(\text{CH}_2\text{RCOOH}) \approx 5$$

which corresponds very closely to the experimentally observed values.^{31,32}

In an extension of these studies^{32,33} it was found that amino acids such as β -alanine and ϵ -aminocaproic do not undergo the reductive deamination reaction 2. It was concluded that e_{aq}^- adds to the C=O bond of the simpler α -amino acids



and that the radical cation intermediate then dissociates



If there is more than one carbon unit between the amino and carbonyl groups reductive deamination does not occur.

The reductive deamination of α -amino acids via reaction 2 was soon confirmed by several additional observations. The free radical transients of reaction 2, $\dot{\text{C}}\text{H}(\text{R})\text{COO}^-$, were observed directly in pulse radiolysis studies of a number of aqueous amino acid systems.³⁴ In this experimental approach the solutions are subjected to an intense pulse (10^{-6} - 10^{-9} sec.) of ionizing radiation and the transient free radicals are monitored and identified by absorption spectroscopy.³⁵ Electron spin resonance (ESR) spectroscopy³⁶ was used in studies of the reactions of photogenerated electrons with α -amino acids in aqueous glasses at low temperatures;³⁷ with this technique it was possible to observe the initial addition product (reaction 2a) at 77K which then dissociated to give the fatty acid radical $\dot{\text{C}}\text{H}(\text{R})\text{COO}^-$ (reaction 2b) on warming to 180K.

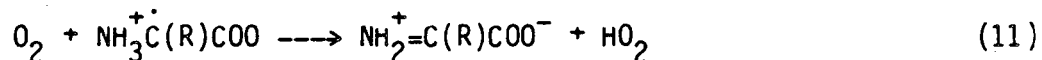
With the aliphatic α -amino acids of higher molecular weight, i.e., with α -amino butyric, valine, leucine, etc., the reductive deamination reaction 2 continues to represent a major path for removal of e_{aq}^- . However, with the longer aliphatic side chains the attack of OH via reaction 3 is no longer confined to the C-H bond at the α -carbon position, other C-H bonds along the side chain also become involved. With the unsaturated α -amino acids such as phenylalanine, tyrosine and histidine, the side chain represents a major competing locus for reaction of both e_{aq}^- and OH.³⁸⁻⁴¹ With the amino acid, cysteine, the reactions of e_{aq}^- and OH occur exclusively at the SH function⁴². Detailed

reaction mechanisms involved in the radiolysis of the various amino acid side-chains are treated in detail in Sec. III.

The presence of dissolved O_2 at a sufficiently high relative concentration results in the blocking of the reductive deamination reaction 2 since the hydrated electron, e_{aq}^- , is preferentially scavenged to yield the hydroperoxy radical, HO_2



where $pK_9 = 4.9$.⁴³ The OH reaction is not inhibited by O_2 and in the case of glycine and alanine the α -carbon radicals $NH_3^+\dot{C}(R)COO^-$ formed in reaction 3 react with O_2 to yield HO_2 and the labile imino acid



which hydrolyses spontaneously to give ammonia and keto acid:



Formation of the hydroperoxyl radical (HO_2 , O_2^-) via reaction 11 has been observed in pulse radiolysis studies of aqueous glycine.⁴⁴

The product stoichiometry in oxygenated solution is approximated by $G(NH_3) = G(\text{Carbonyl}) = G(OH) = 3$.³² The oxidative deamination yield decreases with increasing length of the aliphatic side-chain. With

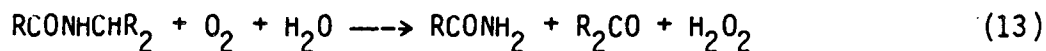
norleucine, $\text{NH}_3^+\text{CH}(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3)\text{COO}^-$, only a small fraction of the OH radicals are removed through H abstraction at the α -carbon position to give $G(\text{NH}_3) \approx 0.3$.

B. Glycine and Alanine Peptides

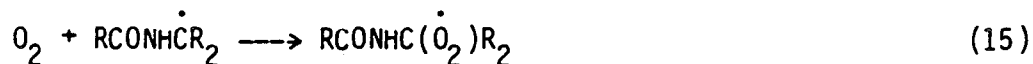
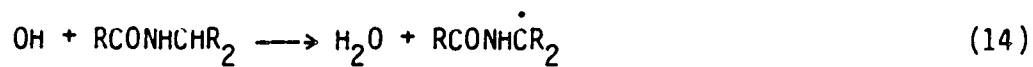
1. Oxygenated Solutions.

(a) N-acetylamino acids

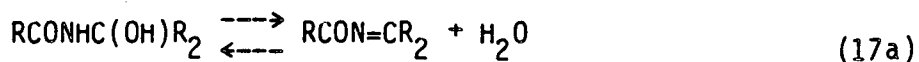
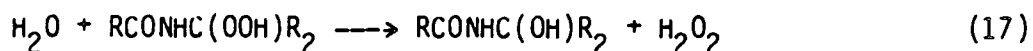
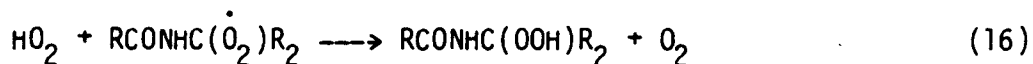
Specific chemical evidence for oxidative degradation of the peptide main chain through OH attack at α C-H bonds to yield amide and keto acid functions was first identified and formulated in terms of the overall stoichiometry.⁴⁵⁻⁴⁷

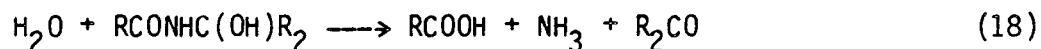


The reaction mechanism proposed at that time includes the radiation induced step 1, 2 followed by:



The subsequent steps were written:

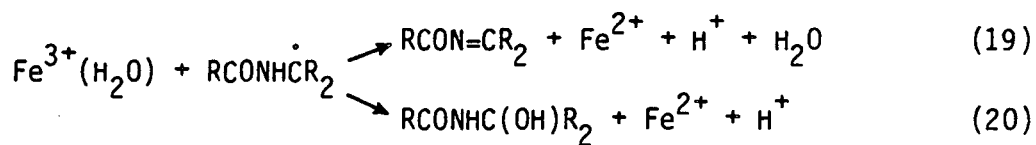




Much of the subsequent information on the detailed mechanisms of oxidative main chain degradation has been derived from studies involving the the peptide derivatives of the simpler α -amino acids glycine and alanine.^{47,48} Radiolysis of N-acetylglycine and N-acetylalanine in oxygenated solution results in the formation of labile peptide derivatives which are readily degraded on mild hydrolysis to yield ammonia and carbonyl products (keto acid plus aldehyde). Free ammonia is not a major initial product in the radiolysis of these systems.

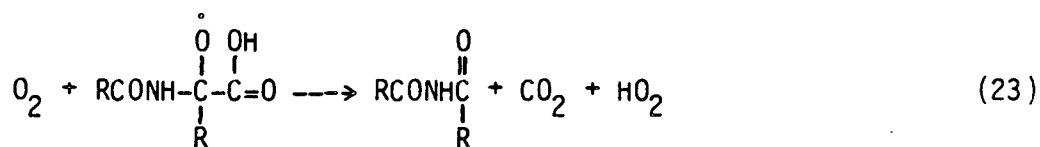
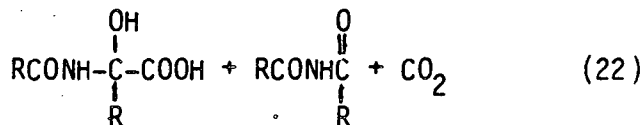
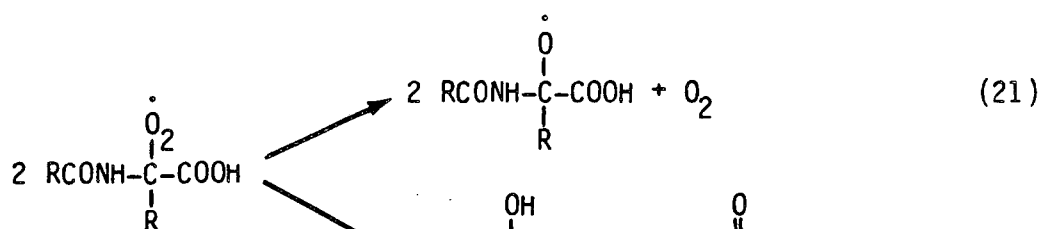
If the radiation degradation of the peptide main chain does occur predominantly through the sequence formulated in eq. 1, 14-18, then it is clear that ammonia and carbonyl yields with γ -rays should be in the relationship $G(\text{NH}_3) = G(\text{R}_2\text{CO}) = G(\text{OH}) = 3$. Quantitative assays of the ammonia and carbonyl yields from a series of model peptide derivatives including acetylglycine, acetylalanine, glycine anhydride, etc., consistently show $G(\text{NH}_3) = 3$ for each system.^{47,48} However, it was also found that the yield of carbonyl products from these simple peptide systems is not in accord with the quantitative requirements of the reaction sequence 14-18. The carbonyl yields (keto acid plus aldehyde) are consistently low with $G(\text{R}_2\text{CO}) = 1$. There was, then, the question as to whether this apparent discrepancy arises from (a) an incorrect formulation of the locus of initial OH attack or from (b) an unspecified complexity in the chemistry of removal of the peroxy radicals $\text{RCONHC}(\dot{\text{O}}_2)\text{R}_2$.

To obtain specific information on these questions, the radiolysis was carried out using ferric ion instead of O_2 as the scavenger of intermediate radicals formed by OH attack on N-acetylglycine and N-acetylalanine.⁴⁸ Heavy metal ions Fe^{3+} and Cu^{2+} oxidize organic free-radicals in aqueous solution by electron transfer and by ligand transfer. Such reactions in the case of the peptide radical $RCONHC\dot{(R)}_2$ would correspond to

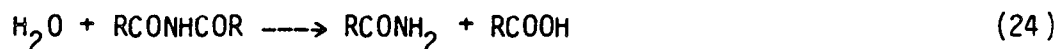


The oxidation products of reactions 19,20 are identical to the postulated products of reactions 16-18 and would then yield amide and carbonyl on hydrolysis. It was found that the γ -radiolysis of 0.1M N-acetylglycine and N-acetylalanine in O_2 -free solution containing 0.05 M Fe^{3+} gives the product stoichiometry $-G(\text{peptide}) = G(NH_3) = G(RCOCOOH) = 3.2 = G(OH) + G(H_2O_2)$. In the presence of Fe^{3+} the values $G(NH_3)$ and $G(RCOCOOH)$ are greater than $G(OH)$ since molecular hydrogen peroxide formed in the radiation step 1 yields additional OH through reaction with Fe^{2+} (generated in steps 19,20) via $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH + OH^-$. The fact that much lower carbonyl yields are obtained with O_2 in place of Fe^{3+} shows then that the chemistry of removal of the peroxy radicals $RCONHC(\dot{O}_2)R_2$ is indeed more complicated than that represented in reactions 15-18.

More complete and detailed examination of the oxidation products formed in the γ -radiolysis of the peptides in oxygenated solutions have established that organic products in addition to keto acid and aldehyde are present.⁴⁷ In the case of N-acetylalanine the organic products identified include pyruvic acid, acetaldehyde, acetic acid and carbon dioxide. The evidence is that the reaction of the peroxy radicals $\text{RCONHC(O}_2\text{)R}_2$ with HO_2 via step 16 occurs in competition with



In the case of N-acetylalanine the diacetamide product, RCONHCOR , of reactions 22, 23 is hydrolytically labile, and under mild differential hydrolysis.



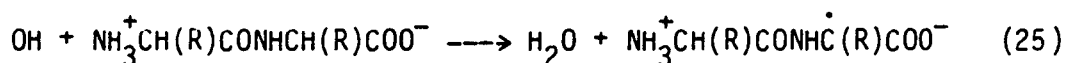
In this way, it was possible to quantitatively separate and measure all of the products formed in the γ -radiolysis of O_2 -saturated N-acetylalanine solutions:⁴⁷

$$G(\text{CH}_3\text{COCO}^-\text{OH} + \text{CH}_3\text{CHO}) \approx 1, G(\text{CH}_3\text{CO}^-\text{OH}) \approx 2.5, G(\text{CO}_2) \approx 2.$$

The formation of several compounds with different oxidation states as initial products is frequently observed in oxidation processes involving peroxy radicals.⁴⁹⁻⁵¹

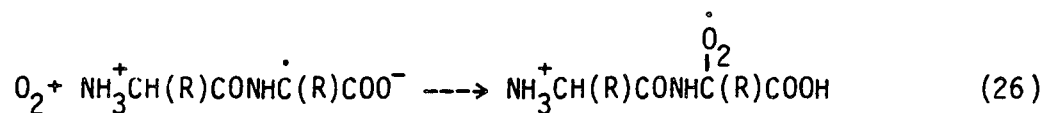
(b) Oligopeptides and polypeptides

The reactions of OH with the dipeptide derivatives of glycine and alanine occur preferentially at the peptide C-H linkage:

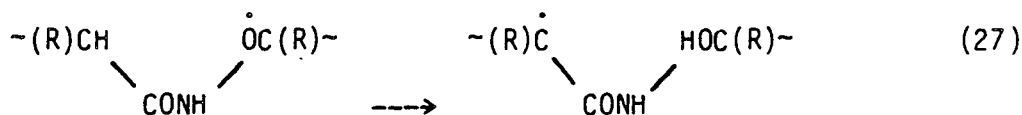


The chemical evidence is that ~90 percent of the OH radicals are so removed.⁵² With the tri, tetra, and poly derivatives the reaction of OH at peptide C-H linkages is essentially quantitative. The kinetics of formation of "peptide" radicals $\sim\text{CONH}\dot{\text{C}}(\text{R})\sim$ through the reaction of OH with simple peptides and oligopeptides via reaction 14 has been extensively studied by pulse radiolysis.⁵³⁻⁵⁵ The reaction of OH at sites along the peptide main-chain (away from the charged terminal residues) appears to be moderately fast with $k_{14} \sim 10^9 \text{M}^{-1}\text{sec}^{-1}$. Since the cyclic dipeptides (amino acid anhydrides) have no end groups they are, in that sense, useful models of interior segments of the polypeptide chain: glycine and alanine anhydrides give k_{14} values of $1.2 \times 10^9 \text{M}^{-1}\text{sec}^{-1}$ and $1.8 \times 10^9 \text{M}^{-1}\text{sec}^{-1}$ respectively.

In the presence of oxygen



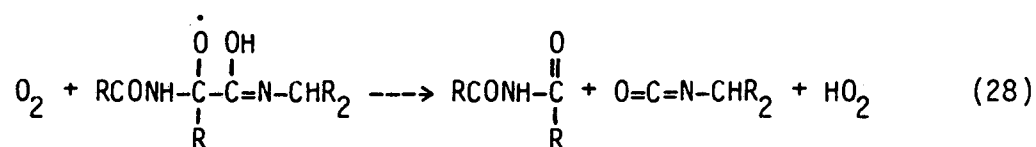
The subsequent chemistry is, in part, quite analogous to that observed in the radiolytic oxidation of N-acetylamino acids via the sequence 14-18, 20, 23 to yield amide and the lower fatty acid. However, with the oligopeptides, the amide yield approaches $G(NH_3) = 5$ which is considerably higher than the value $G(NH_3) = 3 = G(OH)$ observed with the N-acetylamino acids. The chemical evidence is that with oligo and polypeptides an intramolecular reaction occurs.



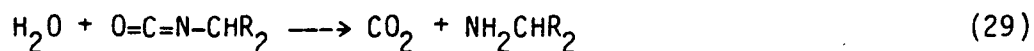
This leads to formation of additional ammonia and keto acid. With diglycine the major product stoichiometries correspond to $G(NH_3) = 4.8$, $G(HCOOH) = 1.7$, $G(CHO_2COOH) = 1.9$.⁵²

With the N-acetylamino acids reaction of type 27 can only occur intermolecularly and is of negligible importance in competition with reaction 23. In agreement with reaction 27 as formulated, an analysis of the carbonyl fraction from a mixed dipeptide, glycylalanine, shows that both keto acids, glyoxylic and pyruvic are indeed formed in approximately equal amounts with a combined yield of $G(>CO) = 2$ with $G(NH_3) = 4.8$.⁵²

Product yields in the γ -radiolysis of polyalanine are consistent with the reaction sequence formulated above, i.e., $G(\text{NH}_3) \approx 4.0$, $G(\text{RCO}_2\text{COOH}) \approx 1.2$, $G(\text{RCOOH}) \approx 3.0$, $G(\text{CO}_2) \approx 2.4$.⁴⁷ With polyalanine at molecular weight ≈ 3000 it must be assumed that alkoxy-radical formation via reaction 14, 15, 23 occurs more or less at random along the peptide chain. The equivalent of reaction 23 then is presumed to involve an adjacent peptide bond (enol form), i.e.,



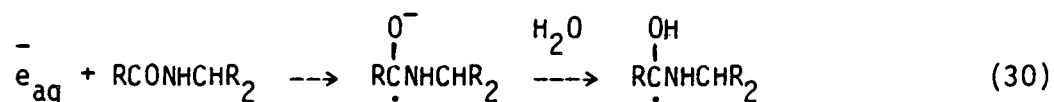
where



follows essentially instantaneously.

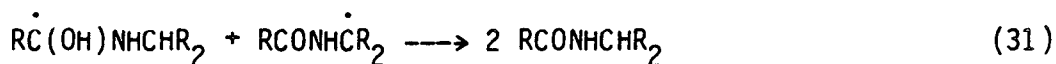
2. Oxygen-free Solutions

The carbonyl group of the peptide bond represents the principle trapping center for e_{aq}^- in oxygen free solutions of peptide derivatives of glycine, alanine and most other aliphatic amino acids.



Chemistry of $\overset{\cdot}{\text{R}}\text{C}(\text{OH})\text{NHCHR}_2$ radicals formed in reaction 30 has been studied in a number of systems including aqueous amides, N-acetylamino acids,^{56,57} and liquid N-ethyl acetamide.⁵⁸ Detailed product-analysis

studies of γ -irradiated N-acetylalanine solutions at pH 7 indicate that the major process for removal of RC(OH)NHCHR_2 radicals involves the back reaction (reconstitution)⁵⁷



where RCONHCHR_2 corresponds to the product of OH attack via reaction 14. It was concluded that main chain cleavage via dissociative deamidation



was relatively unimportant with $G \leq 0.3$ under the experimental conditions employed. Pulse radiolysis studies of the addition of e_{aq}^- to the carbonyl group of the peptide bond do not show any evidence for main chain cleavage via reaction 29.^{55,59,60}

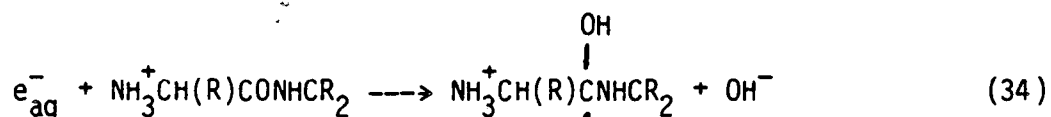
However, ESR studies of the reactions of photogenerated electrons with N-acetyl derivatives of the aliphatic amino acids in aqueous glasses at low temperatures show the addition reaction 30 which is followed by the dissociation reaction 32 as the system is warmed.^{37,61} Reaction 32 has also been observed in a wide variety of aqueous peptide systems through use of spin trapping techniques;⁶² the short lived radical products of reaction 32 are scavenged by an added spin-trap such as t-nitrosobutane



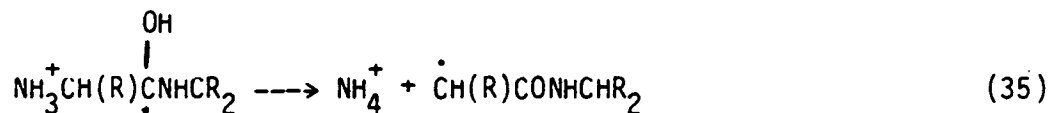
to give longer lived spin-adducts which can be isolated by column chromatography and identified by electron spin resonance (ESR) spectroscopy.

Whether or not the dissociation reaction 32 is observed under a particular experimental condition appears to be related to a dose-rate effect. If the rate of the first order dissociation reaction 32 is relatively slow, then the second order reconstitution reaction 31 would be favored at the higher dose rates. Both the product analysis studies and the pulse radiolysis studies were done at dose rates $\geq 10^{18}$ eV/gm min. The spin trapping studies were done at a dose rate a factor of 10 lower. In the low temperature glasses, dissociation of the "matrix isolated" $RC(OH)NHCHR_2$ radical would be greatly favored over any diffusion controlled recombination reaction.

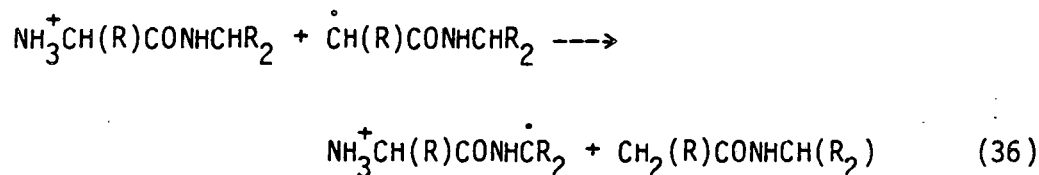
In the radiolysis of the low molecular-weight linear peptide derivatives of glycine and alanine, the addition of e_{aq}^- occurs preferentially at the C=O bond of the N-terminal residue, i.e. for glycylglycine.^{33,63}



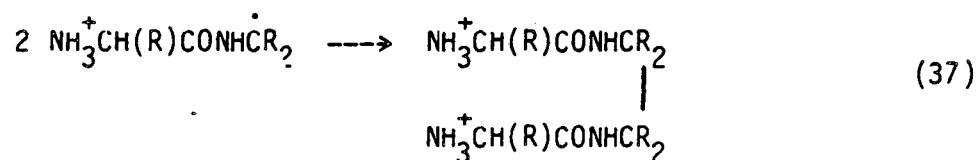
which leads to deamination via



Reactions 34, 35 are characteristic of compounds containing the amino group in the α -position to the carbonyl function i.e., $\text{NH}_3^+\text{CH}(\text{R})\text{COX}$ where $\text{X} = \text{O}^-, \text{OH}, \text{OR}, \text{NHR}$ etc.^{33,34,37,55} Subsequent steps include



and the dimerization reaction:



to yield the α, α' -diaminosuccinic acid derivatives.^{26,63,64}

The yields of the reductive deamination reaction 34,35 with di and triglycine corresponds to $G(\text{NH}_3) \approx 3 = e_{\text{aq}}^-$. The free ammonia yield from tetraglycine is somewhat lower, $G(\text{NH}_3) \approx 2.4$. With polyalanine the free ammonia yield decreases to $G(\text{NH}_3) \approx 0.3$: This decrease in free ammonia is accompanied by an increase in "amide" cleavage resulting from capture of e_{aq}^- by $\text{C}=\text{O}$ bonds away from the terminal residue via reactions 27,29.^{61,62} Rate measurements for reactions of e_{aq}^- with oligopeptide and cyclic dipeptide derivatives of glycine and alanine indicate^{55,60} that addition of e_{aq}^- to an "internal" peptide bond is of moderate rate with $k_{30} \sim 1 \times 10^9 \text{M}^{-1}\text{sec}^{-1}$.

III. Side-Chain Chemistry of Peptides in Aqueous Solution

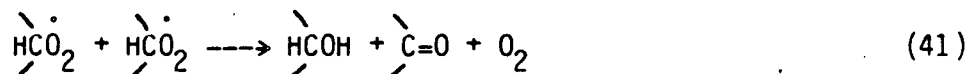
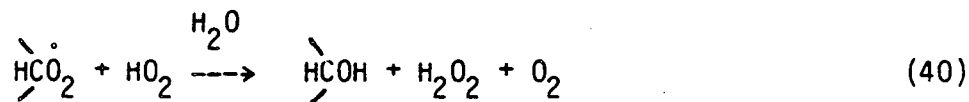
A. Aliphatic Residues

1. Oxygenated Solutions

Although OH attack at the glycine and alanine residues occurs almost exclusively at the α C-H position along the peptide main chain (reaction 14), with all other aliphatic amino acids the side chain represents a major competing locus of OH reaction 46,65,66. With the alkyl series, α -aminobutyric, valine, leucine, etc., the yield for oxidative degradation of the main chain to yield amide, keto acid and fatty acid functions (Sec. II.B) decreases with increasing number of C-H bonds in the hydrocarbon chain.^{32,67} Competing side-chain chemistry leads to hydroxyl and carbonyl substitution via

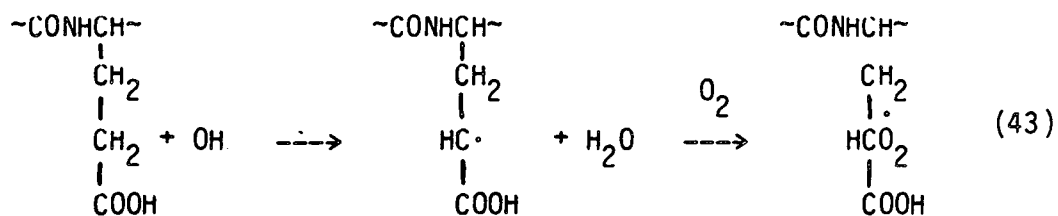


followed by the characteristic reactions of alkyl peroxy radicals.⁴⁸⁻⁵¹

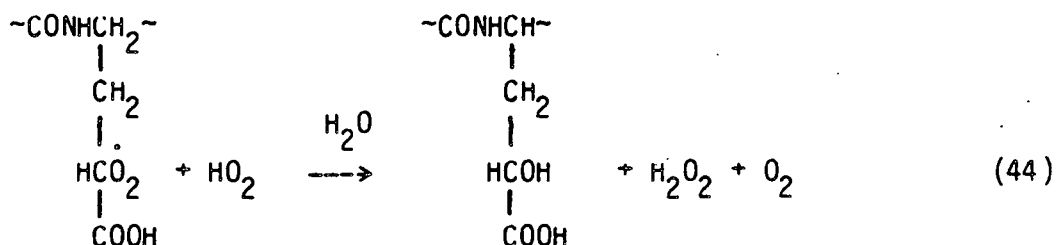


Oxidation can occur at any C-H position along the chain. Valine and leucine yield 3-hydroxy valine and 3-hydroxy plus 4-hydroxy leucine respectively as major products. Detailed chemical identifications and quantitative determinations of the various hydroxy and carbonyl products formed through OH attack at side chain loci of α -aminobutyric, valine and leucine have been made.⁶⁶

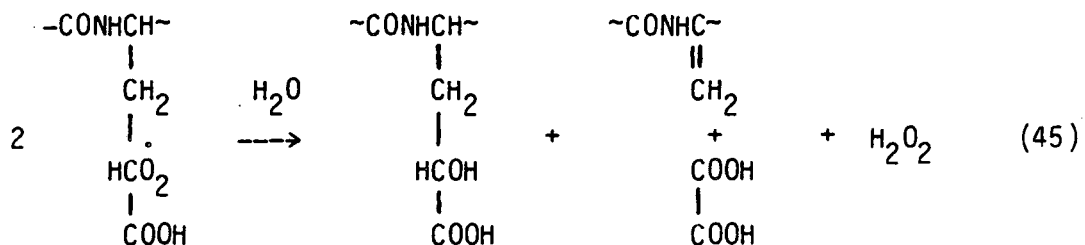
With the dicarboxylic amino acids aspartic and glutamic the C-H bond α to the side-chain carboxyl group represents a principal locus of OH attack in competition with the main-chain reaction 14 as observed by both product analysis⁶⁸ and spin trapping methods.⁶⁵ With N-acetylglutamic acid in oxygenated solution, main chain degradation via reactions 14-18 to yield amide and α -keto-glutaric acid functions accounts for ~30 percent of the OH radicals.⁶⁸ The remainder attack at the side chain via



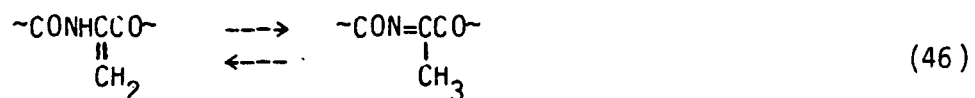
Part of the subsequent chemistry ($\text{RO}_2 + \text{HO}_2 \longrightarrow$) is similar to that formulated in equation 40 i.e.,



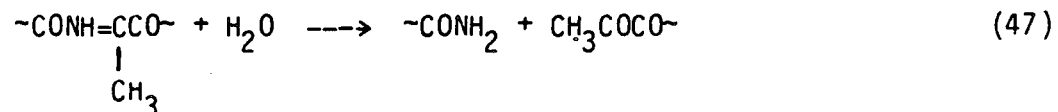
However, the analogues of the competing reactions ($2R_2O_2 \rightarrow$) takes a more complicated form than that shown in equations 41,42 i.e.,



Reactions akin to the degradation reaction 45 have been observed in other systems.⁴⁹ The unsaturated degradation product formed in reaction 45 corresponds to a class of compounds referred to as dehydropeptides.



These compounds are easily hydrolysed to yield amide and keto acid functions (cf eq. 17-18)



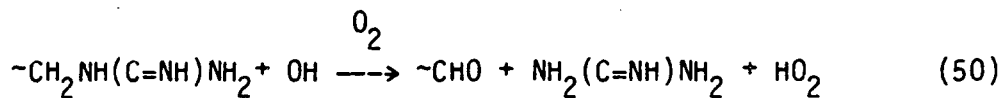
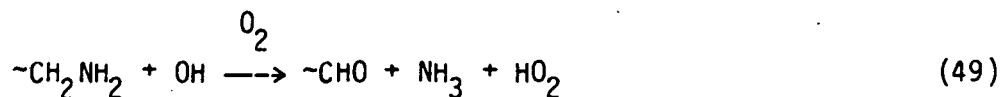
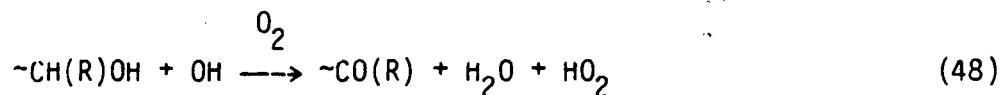
We have here an example of a case in which OH attack at a side chain locus can lead to oxidative degradation of the peptide main chain.

Product yields in the γ -radiolysis of N-acetylglutamic acid (0.1 M, O_2 -sat) are essentially independent of pH over the range pH 3 to 8 with $G(\text{amide}) = 2.3$, $G(\alpha\text{-keto-glutaric}) = 0.8$, $G(\text{pyruvic}) = 0.9$. Similar product yields are obtained with polyglutamic acid solutions (0.15 M, O_2 -sat) over the pH range 6-8. But, the amide and pyruvic acids yields from PGA decrease abruptly as the pH is lowered from 6 to 4 whereas the α -keto-glutaric yield remains essentially constant over the entire pH range 8 to 3.

In interpreting these pronounced pH effects in the polyglutamic system, it has been pointed out that one of the unique characteristics of the radiation chemistry of macromolecular substances in aqueous solution is that each (macro)molecule undergoes reaction with a relatively large number of OH radicals even at the lowest practicable dosages.^{67,68} For example, with a 0.15 M solution of PGA, a γ -ray dose of 3×10^{18} eV/g produces only one OH per 100 glutamic acid residues, but, at the same time this corresponds to about 20 OH radicals per PGA molecule (MW 140,000). Since PGA above pH 6 has the random coil configuration, the various segments of the macromolecule are free to interact both intermolecularly and intramolecularly, and we find at pH > 6 no essential differences between the macromolecule and the low molecular weight model from the standpoint of product yields. But, as the pH of the solution is decreased, PGA undergoes a coil \rightarrow helix transition over the pH range 6 to 4.5. This is the range over which there is an abrupt decrease in the amide and pyruvic acid yields. With PGA in the helix form, the peroxy radicals $\dot{R}O_2$ are

frozen in a fixed spatial arrangement and it is obvious that the probability of reaction 45 ($2\text{RO}_2 \rightarrow$) is greatly reduced, reaction 44 ($\text{RO}_2 + \text{HO}_2 \rightarrow$) is favored and as a result the yield of main chain degradation decreases as observed experimentally.^{67,68}

The loci of OH attack with asparagine and glutamine residues are analogous to those observed with peptide derivatives of the parent compounds aspartic acid and glutamic acid as formulated above.⁶⁵ Serine, threonine, lysine and arginine undergo oxidation at the α C-H position via reaction 14 etc., in competition with their respective side-chain reactions some of which yield carbonyl products via reactions akin to those given in eqs. 11, 12 above e.g.



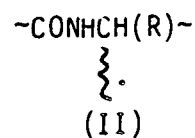
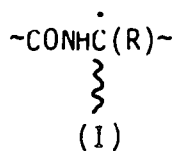
plus products of higher oxidation.

The reactions of OH radicals with peptide derivatives of the aliphatic amino acids outlined above, all involve the formation of carbon centered radicals through H abstraction. Such reactions are of intermediate velocity with k values^{60,69} in the range $1-5 \times 10^9 \text{M}^{-1}\text{sec}^{-1}$.

2. Evacuated Solutions

The available data from product analysis,⁶⁷ pulse radiolysis^{55,60} and spin trapping studies⁶² in irradiated aqueous solutions and from ESR studies⁶¹ of photogenerated electrons in aqueous glasses indicate that the carbonyl bond of most aliphatic amino acid residues represents the major trapping center for e_{aq}^- via the addition reaction 30. This applies to (a) the alkyl amino acids, glycine, alanine, valine, leucine etc., (b) the dicarboxylic acids, aspartic, glutamic and their respective amides, asparagine and glutamine, (c) the basic amino acids, lysine and arginine, (d) the hydroxy containing amino acids, serine and threonine. These addition reactions are all of intermediate velocity with k values in the $10^8 - 10^9 \text{ M}^{-1} \text{ sec}^{-1}$ range.^{60,70}

In the absence of oxygen, both main chain and side chain radicals



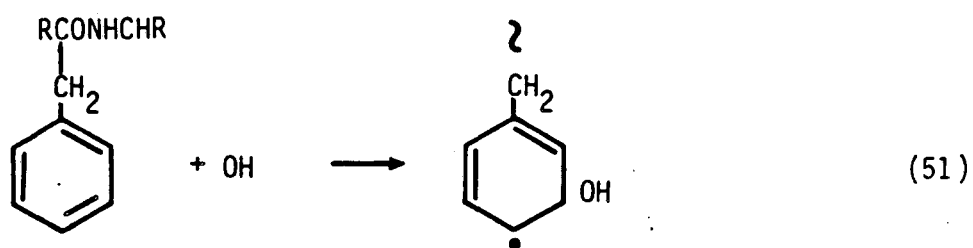
formed through OH attack in these systems undergo dimerization to yield, α, α' -diaminodicarboxylic derivatives.²⁶ The dimerization of type I radicals from glycine and alanine residues to give α, α' -diamino-succinic and α, α' -diaminodimethyl succinic acids respectively has been discussed. Higher molecular weight dimers such as α, α' -diaminopimelic and α, α' -diaminosuberic formed through dimerization of type II radicals have also been identified.⁶⁶ The formation of unsaturated dimers through combination of side chain radicals derived from aromatic-unsaturated amino acid residues is discussed below.

B. Aromatic Unsaturated Residues

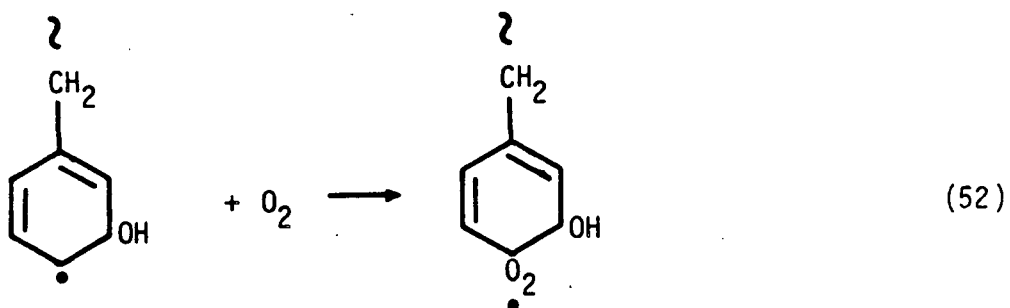
1. Oxygenated Solutions

Reactions of OH radicals with the phenylalanine residue include:

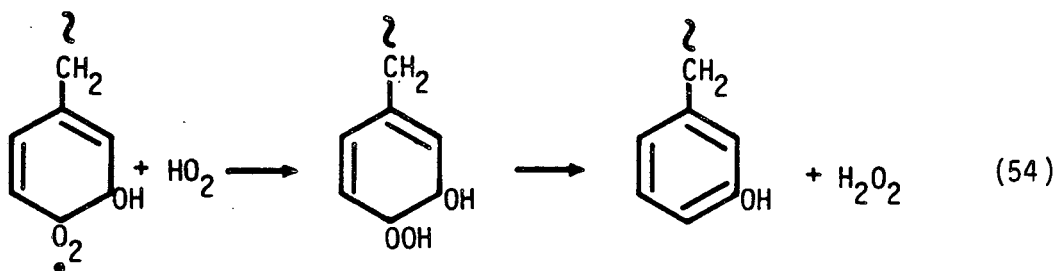
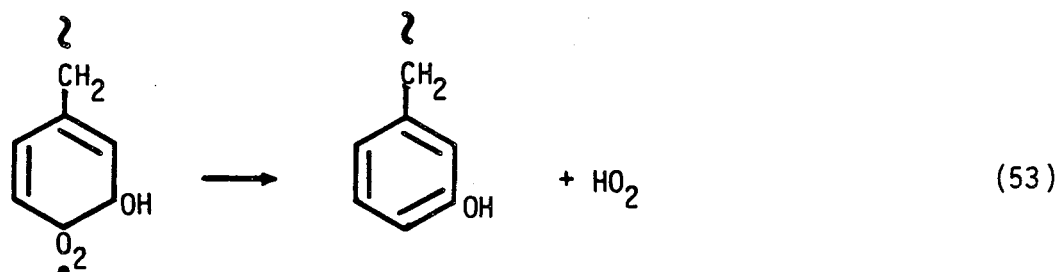
(1) H-abstraction at the main chain via reaction 14 to give the main-chain radical⁶⁵, $\text{RCONH}\dot{\text{C}}(\Phi)\text{R}$, (2) addition to the aromatic side-chain.^{71,72}



where reaction 51 represents the principal path for OH removal. In the presence of O_2 , the main chain radicals, $\text{RCONH}\dot{\text{C}}(\Phi)\text{R}$ undergo oxidative degradation with formation of amide, phenylpyruvic acid and products of higher oxidation through reactions analogous to those formulated in equations 14–23. The hydroxycyclohexadienyl radicals formed through the OH addition reaction 51 react with O_2 to yield peroxy radical intermediates



which undergo the subsequent reactions

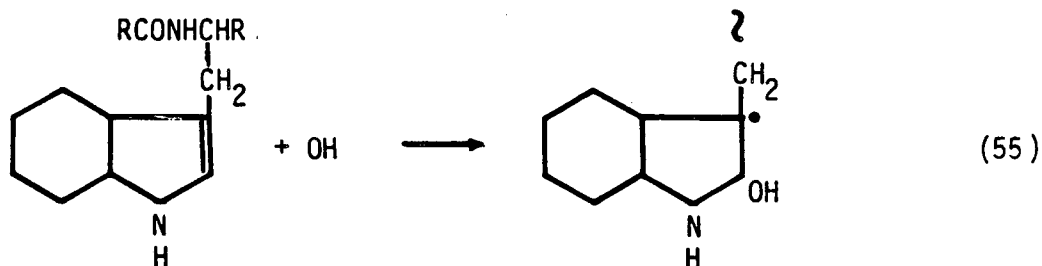


to yield tyrosine (ortho, meta, para) as the major products.⁷¹⁻⁷³ In the radiolysis of aqueous benzene a fraction of the peroxy radicals formed in reaction 52 undergo rearrangement and further oxidation to yield β -hydroxymucondialdehyde.⁷³

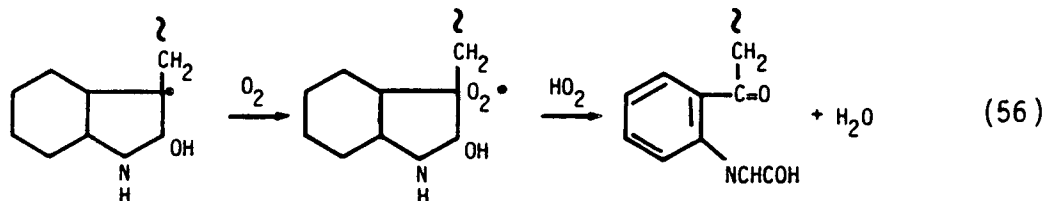
The radiolytic oxidation of tyrosine residues in O_2 saturated solution appears to involve reactions analogous to those given in equations 51-54 to yield dopa, 3,4-dihydroxyphenylalanine, plus other unidentified products.^{74,75}

The radiolytic oxidation of the tryptophan residue in oxygenated solution arises predominantly through reactions initiated by OH

addition to unsaturated bonds of the indole moiety as evidenced by both product analysis and pulse radiolysis studies.^{39,76,77} The addition of OH to the C2-C3 double bond of the indole heterocyclic ring e.g.,

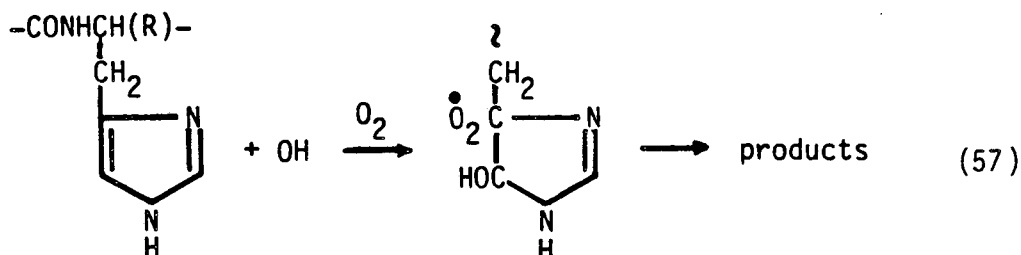


leads to formation of formylkinurenine as a major degradation product in oxygenated solution.⁷⁶



The addition of OH to the benzenoid ring leads to formation of phenolic products in lesser yield. A more recent pulse-radiolysis study using a computerized analysis of the transient absorption spectra suggests that about 40 percent of the OH radicals add to the aromatic ring and about 60 percent react at the C₂-position⁷⁸ (eq. 55). The attack of OH radicals at the peptide main-chain appears to be minimal in this system.

Product analysis,⁷⁹ ESR,⁸⁰ and pulse radiolysis^{81,82} studies all show that the major mechanism for OH attack at the histidine residue involves addition to the imidazole ring e.g.,

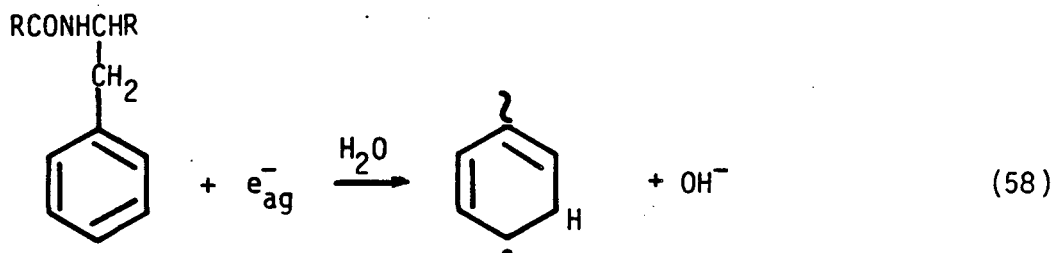


The yield for oxidative degradation of the histidine (imidazole) ring corresponds to $G(-\text{His}) \sim 4$ in dilute O_2 -saturated solutions under γ -rays and a complexity of degradation products are observed.⁷⁹ Among the major products of reaction (57) are asparagine and aspartic acid. The identification of imidazolylpyruvic and imidazolylacetic acids as lesser products indicates that OH attack at the main chain via reaction 14 (followed by the analogues of reactions 14-23) is also involved in the reaction of OH radicals at the histidine residue.

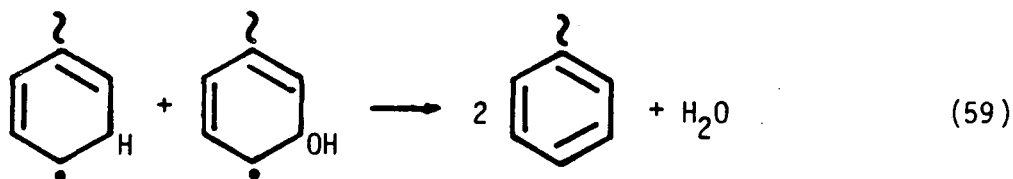
The reactions of OH radicals with the phenylalanine, tyrosine, tryptophan and histidine residues are relatively fast with k -values in the $5 \times 10^9 - 10^{10} \text{ M}^{-1} \text{ sec}^{-1}$ range.^{60,69}

2. Oxygen free Solutions

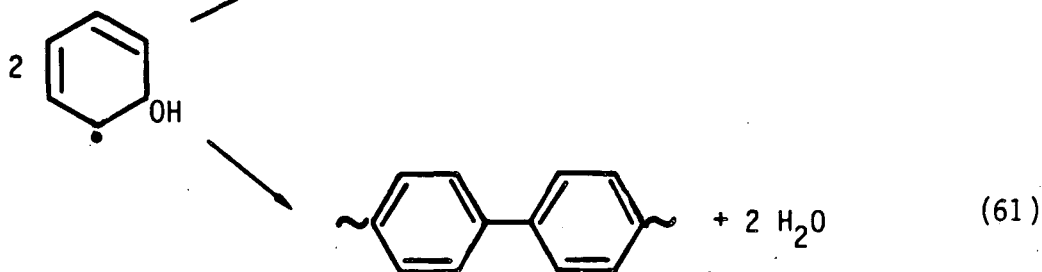
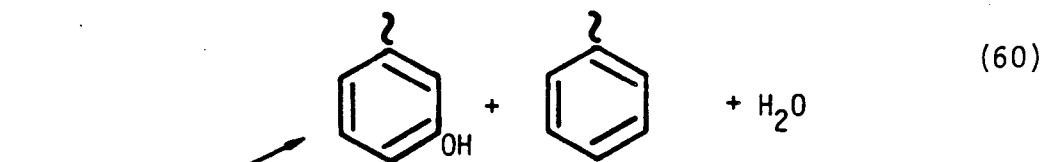
In oxygen free solutions, both the peptide C=O bond and the benzene ring of the phenylalanine residue represent major trapping centers for the hydrated electron, e_{aq}^- , via reaction 30 and the reaction 58.^{61,62,82}



Pulse radiolysis studies of aqueous N-acetylphenylalanine indicate that ~50 percent of e_{aq}^- reacts via addition to the peptide C=O bond, the remainder add to the benzene ring via reaction 58.⁸² Subsequent reactions of the $e(\text{H})$ adduct⁸³ formed in step 58 and the OH adduct of step 51 above include the back-reaction

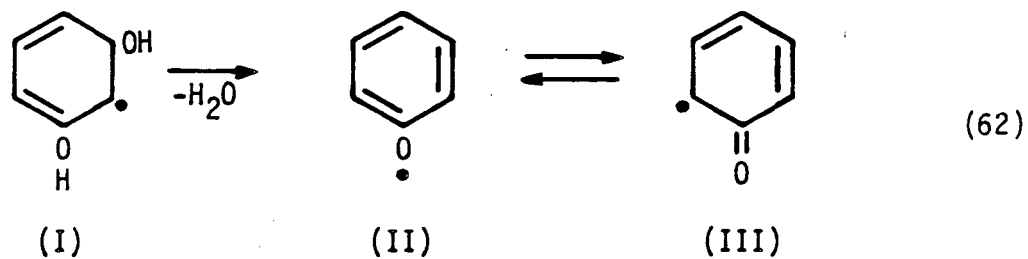


and

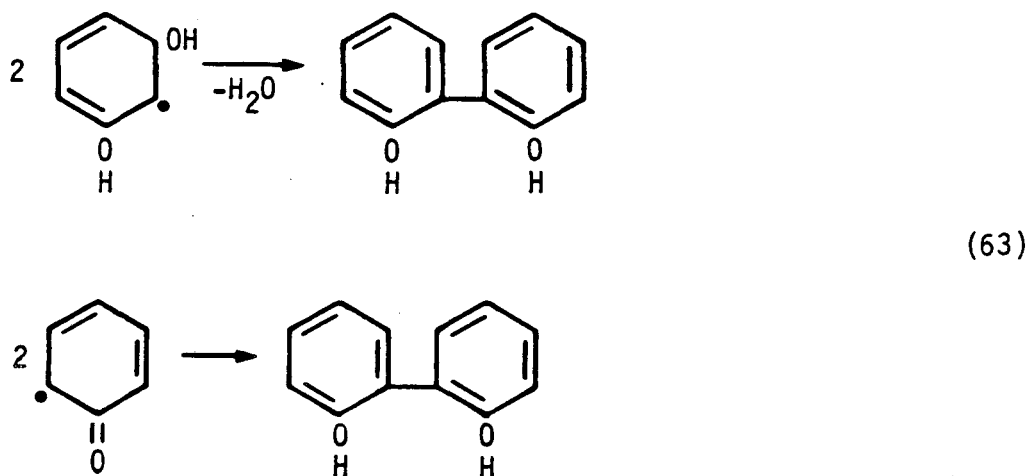


to give tyrosine, phenylalanine dimer and their respective isomers.⁸⁴⁻⁸⁶ Detailed gas chromatography-mass spectrometry (GCMS) studies of product yields in the γ -radiolysis of phenylalanine and its homopeptides in oxygen-free N_2O -saturated solution give G values of 0.50, 0.30, 0.5 and 1.5 for the formation of o-,m-,p-, tyrosines (eq. 60) and dimers (eq. 61) respectively.⁸⁶ Other direct experimental evidence for the radiolytic production of biphenyl type dimers from aqueous phenylalanine peptides has been reported.⁸⁷⁻⁸⁹ Studies of OH mediated crosslinking between ^{14}C -labelled phenylalanine and a phenylalanine containing peptide, Gly-Gly-Phe-Leu, indicate that the observed tetrapeptide-phenylalanine adducts are formed predominantly through Phe-Phe linkages.⁸⁸

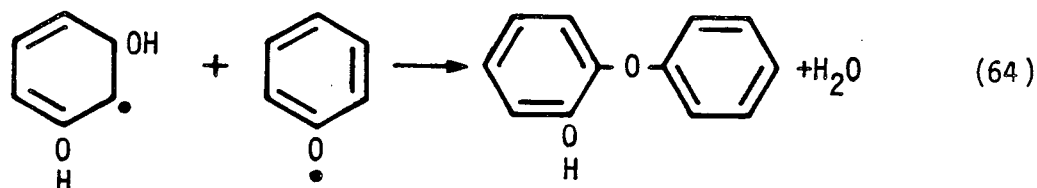
Chemistry similar to that given in reaction 60, 61 also occurs in the radiolysis of aqueous solutions of tyrosine and tyrosine peptides. A blue fluorescent product characteristic of dityrosine is observed in irradiated oxygen-free solutions of tyrosine, glycylyltyrosine and polytyrosine.^{90,91} More recent studies⁹² of the γ -ray induced crosslinking of tyrosine peptides in N_2O saturated solution have employed GCMS techniques for the isolation and identification of dimer products. The findings show that crosslinking occurs through both C-C and C-O-C bonding. The evidence is that the reaction of OH with tyrosine via the analogue of reaction 51 yields dihydroxydienyl radicals and that a fraction of these dienyl radicals, I, are present in the phenoxy form, II, which is in resonance with radical III.^{90,92}



Dimerization of radicals I and/or III yield dityrosine

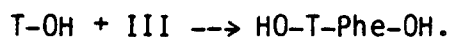
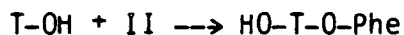


while combination of radicals I, III with the phenoxy radical II yields the observed diphenyl ether derivative.



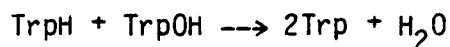
A G value of 1.5 has been reported for the combined yield of these dimer products.

The GCMS approach has also been applied in studies of the cross-linking of tyrosine and the DNA base thymine (T) in γ -irradiated N_2O saturated solution. The reaction of OH with T occurs predominantly through addition to the C(5)=C(6) double bond to give the TOH radical. When both TOH and tyrosine radicals II, III (eq. 62) are present simultaneously, both C-C and C-O-C crosslinks are formed i.e.



The combined yield of T-Tyr dimers corresponds to $G \sim 1.0$.⁹²

Pulse radiolysis and ESR studies indicate that both e_{aq}^- and OH react with tryptophan almost exclusively through addition to the indole moiety.^{39,76,93} The γ -ray yield for tryptophan destruction $G(-\text{Trp})$ is extremely low in O_2 -free solution. The reconstruction reaction



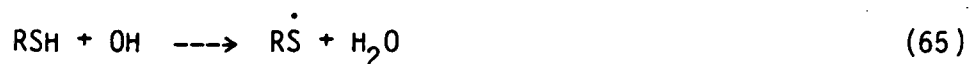
represents the principal radical-removal step in the absence of O_2 .

The protonated imidazole ring of the histidine residue reacts very rapidly with e_{aq}^- ($k \sim 5 \times 10^9 \text{ m}^{-1} \text{ sec}^{-1}$) to give a radical product with two characteristic absorption bands $\lambda_{\text{max}} = 290$ and 360 nm .^{60,81} The non-protonated ring is much less reactive towards e_{aq}^- ; with a simple peptide such as glycylhistidine at $\text{pH} > 7.8$, e_{aq}^- adds preferentially to the peptide carbonyl bond via reaction 34 which is followed by the deamination reaction 35.^{60,94}

C. Sulfur-Containing Residues

1. Oxygenated Solutions

The reaction of OH at the cysteine residue occurs preferentially at the sulfur moiety



where reaction 65 is essentially diffusion controlled with $k = 10^{10} \text{ M}^{-1} \text{ sec}^{-1}$.^{60,69} In acidic oxygenated solution the overall stoichiometry corresponds to:⁹⁵⁻⁹⁸



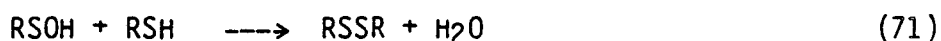
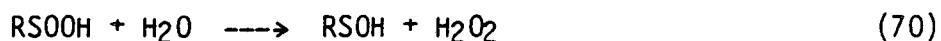
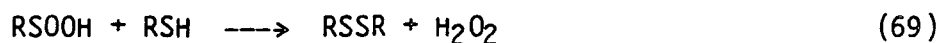
In the γ -radiolysis of 10^{-3} M cysteine in O_2 -saturated solution at $\text{pH} 3$, $G(-\text{RSH}) = 10$, $G(\text{RSSR}) = 5$, $G(\text{H}_2\text{O}_2) = 5$. The mechanism in acidic solution involves a short chain:



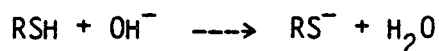


The hydroperoxide radical, HO_2^\bullet , appears to be unreactive towards RSH.

Observed products are formed via the subsequent chemistry



A marked increase in the values of $G(-\text{RSH})$, $G(\text{RSSR})$, $G(\text{H}_2\text{O}_2)$ is observed with increasing pH above $\text{pH} \geq 5$.^{96,98} The evidence is that the thiolate ion



competes with oxygen (reaction 67) for thiyl radicals via



where the radical ion reacts in turn with oxygen



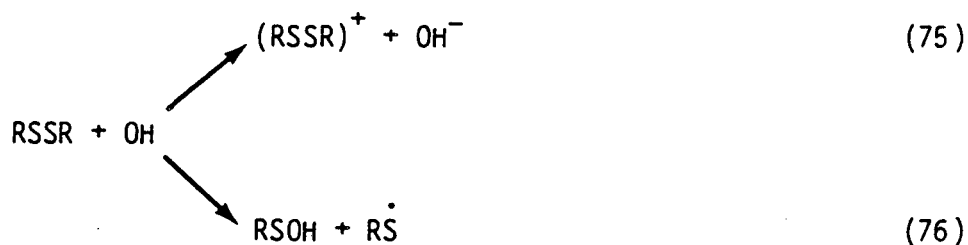
which generates additional RS^\bullet via⁹⁸



Reactions 72-74 constitute a chain which gives $G(-\text{RSH})$ values as high

as 50 in the γ -radiolysis of 3×10^{-3} M cysteine containing 2×10^{-4} M oxygen. Although H_2O_2 as noted above does not react with RSH, the conjugated base O_2^- (eq. 9) does, with $k_{74} = 1.8 \times 10^4 \text{ M}^{-1} \text{ sec}^{-1}$.

Oxidation of the disulfide linkage of cystine by the hydroxyl radical is also a fast reaction ($k = 1 \times 10^{10} \text{ M}^{-1} \text{ sec}^{-1}$) which involves ion-pair formation and dissociative OH addition.⁹⁹⁻¹⁰¹



where reactions 75,76 occur with about equal probability. In the presence of O_2 , the principle final oxidation products, the sulfuric and sulfonic acid derivatives, RSO_2H and RSO_3H are formed in the γ -radiolysis of 10^{-3} M cystine with $G = 1.7$ and $G = 0.7$ respectively.¹⁰²

Chemical and pulse radiolysis studies show that methionine in the peptide form reacts with OH primarily as a thioether.⁹⁹ Oxidative attack occurs predominantly at the sulfur locus to give an addition product ($k_{77} \sim 10^{10} \text{ M}^{-1} \text{ sec}^{-1}$)



which in oxygen-free solution breaks down in a complex series of reactions to yield a cation dimer and a sulfone.^{99,103} Products derived from OH-induced cleavage of the methionine C-S-C linkage are formed in small amounts with a combined yield of $G \sim 0.6$.¹⁰⁴ In oxygenated

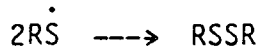
solution the major final products of γ -radiolysis are methionine sulfone, $RS(O)CH_3$, and methionine sulfoxide, $RS(O_2)CH_3$, with a combined yield of $G = 3.10^5$

2. Oxygen-free Solutions

The reaction of e_{aq}^- at the cysteine residue



is essentially quantitative with $k_{78} = 10^{10} \text{ M}^{-1} \text{ sec}^{-1}$ in neutral solution. The e_{aq}^- reaction 78 and the OH reaction 65 lead to the subsequent chemistry



to give $G(\text{cystine}) = G(\text{alanine}) = G(\text{H}_2\text{S}) = 3$ in steady-state γ -radiolysis.^{42,106} Pulse radiolysis studies¹⁰⁷ are in accord with the above formulation.

The reactions of disulfides, cystamine, cystine etc. with e_{aq}^-

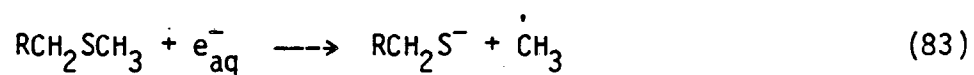
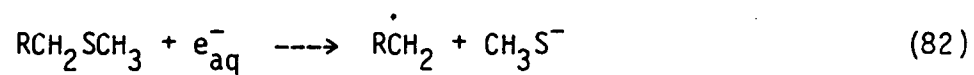


are also fast with $k_{80} \sim 10^{10} \text{ M}^{-1} \text{ sec}^{-1}$. The anion radical product absorbs strongly with a band centered at $\sim 410 \text{ nm}$ with an extinction of $\sim 10^4 \text{ M}^{-1} \text{ sec}^{-1}$. The first order constant for the decay reaction



is independent of pH in the range 4-7.5 but rises sharply with decreasing pH.^{108,109}

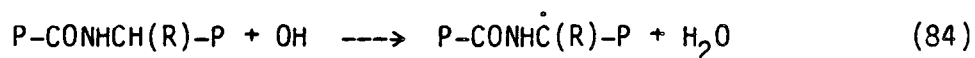
With methionine and its peptide derivatives the reaction of e_{aq}^- occurs preferentially at the main-chain C=O bond via the addition reaction 30. ESR studies of spin-trapped (tNB) radicals also indicate a small contribution of⁶⁵



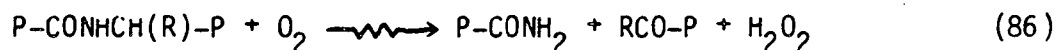
IV. Main-Chain Chemistry of Proteins in Aqueous Solution

1. Oxygenated Solutions

The early studies of the oxidative degradation of the peptide chain in aqueous O_2 -saturated solutions^{45,46} involved both protein and model peptides systems. The reactions of OH at C-H positions along the protein main-chain were found to lead to oxidative degradation via



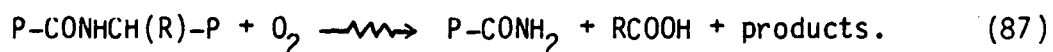
followed by analogues of reactions 16-18 to yield amide and keto acid functions. The overall chemistry of this reaction sequence is the stoichiometric equivalent of equation 13 (Sec. II) i.e.



The attack of OH via reaction 84 was viewed as occurring in competition with OH attack at side-chain loci, with the yield of main-chain degradation being affected by both the amino acid composition and the conformational characteristics of the protein.

A detailed study of keto acid and amide production in the γ -radiolysis of soluble collagen (gelatin 1 percent in oxygenated solution) was included in this early study. The keto-acid products include: glyoxylic, pyruvic, phenylpyruvic α -keto-glutaric, oxalacetic plus

traces of other unidentified α -keto acids to give a combined carbonyl yield of $G(>CO) \approx 0.9$. The combined amide yield is appreciably higher with $G(\text{amide}) \approx 1.25$. More detailed studies with model peptides^{47,52} show that the observed difference between $G(>CO)$ and $G(\text{amide})$ arises in part from the fact that a second (parallel) chemistry is involved in the radiolytic degradation of the protein main-chain.^{46,47,52} This second degradation mode yields the amide function and organic products of higher oxidation:



The specific intermediate chemistry is given in Sec II B.

The separation and isolation of protein fragments formed by main-chain cleavage in the X-radiolysis of ribonuclease (RNase), bovine serum albumen (BSA), and lactate dehydrogenase (LDH) in oxygenated solution has recently been achieved.^{110,111} The protein fragments become apparent only after reduction of S-S bonds in the irradiated proteins with mercapto ethanol (RSH)



in aqueous sodium dodecyl sulfate, SDS. Separation was achieved by a combination of gel filtration and gel electrophoresis. The observed

fragmentation yield (G value) for RNase, BSA and LDH are ~ 0.19, 0.3, 0.1 respectively. Proteins, such as LDH, which contain larger amounts of aromatic-unsaturated and sulfur-containing amino acids would be expected to show lower main-chain fragmentation yields. These values could be somewhat low as a measure of OH radical attack at the protein main-chain in these systems. Oxidative main-chain cleavage represented by equation 86 involves the hydrolysis of dehydropeptide intermediates as outlined in the reaction sequence 14-18. While these intermediates are known to be labile, there is no evidence that the cleavage reaction 18 occurs spontaneously. Mild acid hydrolysis prior to the filtration and electrophoresis steps could result in fragmentation yields somewhat greater than those reported.

Pulse radiolysis and kinetic spectroscopy studies have also provided evidence for OH reactions at the protein main-chain. The $\sim\text{CONHC}(\text{R})\sim$ radical in model peptides has been shown to have a strong absorption band at 250 nm with a relatively high extinction coefficient.^{55,59} Studies of the absorption spectra produced by OH attack on papain¹¹² and ribonuclease¹¹³ suggest that ~20-30 percent of the OH radicals generated in these systems react at C-H positions along the protein main-chain.

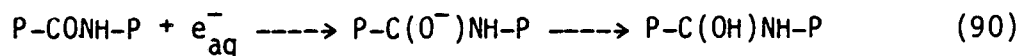
Combined gel filtration and ultracentrifugation studies of proteins irradiated in oxygenated solution have shown the presence (before reduction with RSH) of conformationally changed monomers with a lower γ -helical content than the "native" protein.^{114,115} This radiation-induced unfolding of the protein structure is observed only in oxygenated solution and has been followed in a number of protein systems by

measuring changes in optical rotatory dispersion,¹¹⁵ Amide I absorption¹¹⁶ and tryptophan fluorescence.¹¹⁷ These authors have suggested that this radiation induced disruption of the protein helix in oxygenated solution arises from chemical change at side-chain sites. It would seem that oxidative main chain breaks via the chemistry of equations 86, 87 (which occur in parallel with oxidative changes at side-chain sites) could also be an important factor in the destabilization of the α -helical structure of proteins irradiated in oxygenated solution.

2. Oxygen-Free Solutions

The carbonyl group of the peptide bond represents a major trapping center for e_{aq}^- via reaction 30 in oxygen free solutions of simple peptides^{56,59} and more complex polypeptides.^{62,118} The adduct products have weak absorptions in the spectral region below 300 nm and are relatively long lived.⁵⁹

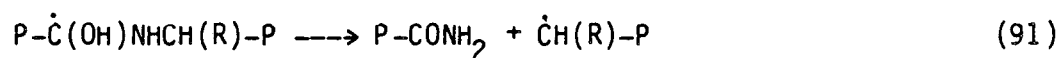
Various types of evidence indicate that the addition of e_{aq}^- to the peptide carbonyls of proteins in oxygen free solutions



occurs in competition with e_{aq}^- addition to the disulfide bond of cystine (sec III C) and the protonated histidine residue (sec III B). For example, pulse radiolysis studies of papain¹¹² and α -chymotrypsin¹¹⁸ indicate that reaction 90 represents a major path for removal e_{aq}^- in these systems with the remainder being trapped predominantly at cystine

disulfide linkages and at histidine residues.¹¹⁹ On the other hand, with lysozyme and trypsin,^{120,121} e_{aq}^- is preferentially trapped at cystine residues.

Recent spin-trapping and ESR studies¹²² of the reactions of e_{aq}^- with the chromosomal proteins histone H1 and protamine (and the related compounds polyglycine, polyarginine and polyalanine) indicate that the dissociation reaction 91 (the analogue of reaction 32, Sec. 11)



represents a major chemical consequence of the addition of e_{aq}^- to peptide carbonyls of histone H1 and prolamine (and the polyamino acid derivatives of lysine, arginine and alanine). Polyacrylamide gel electrophoresis of the irradiated protamine and histone show extensive main-chain scission although quantitative yield data are not reported. It is to be noted that histone H1 (calf thymus) and protamine (salmine) contain no cysteine, cystine, methionine or histidine which are known to be effective as competing centers for electron capture.

It has been suggested^{60,123} that e_{aq}^- first adds to peptide carbonyl bonds located on the surface of the protein and that electron transfer from $-C(O^-)NH-$ radicals to cystine and histidine residues occurs through hydrogen bonds between peptide units.¹²⁴ Recent ESR studies of metallo-proteins in γ -irradiated aqueous glasses at 77K suggest that such electron transfer from one peptide unit to the next is a fast process.¹²⁵ It is also of interest in this regard to note

the finding that the mobilities of electrons and holes in "dry" polyglycine are $\sim 1.5 \text{ cm}^2/\text{Vs}$ and $\sim 6 \text{ cm}^2/\text{Vs}$ respectively whereas with "hydrated" polyglycine the corresponding values are $\sim 150 \text{ cm}^2/\text{Vs}$ and $\sim 4 \text{ cm}^2/\text{Vs}$, indicating that electrons in the "hydrated" polyglycine can be considered as conventional delocalized charge carriers.¹²⁶

V. Side Chain Chemistry of Proteins (and Nucleoproteins)
in Aqueous Solution

A. Sulfur-containing Residues

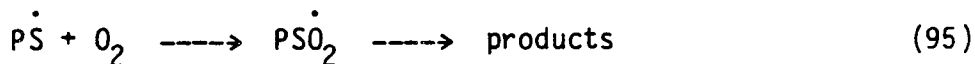
Studies with papain^{112,127} and glyceraldehyde -3- phosphate dehydrogenase, GAPDH,¹²⁸ have established three reaction modes for the radiation induced oxidation of protein SH groups in oxygenated solutions i.e.



and the slower molecular process

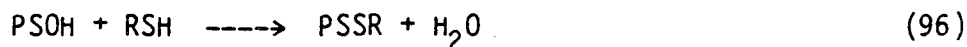


The radical reactions 92,93 which form the protein $\dot{\text{P}}\text{S}$ radicals are analogues of reactions 65,74 which were identified in radiation chemical studies of cysteine and its simple model peptides (Sec III C). Subsequent reaction of macromolecular $\dot{\text{P}}\text{S}$ with O_2



leads to formation of non-repairable products which are presumed to be the sulfinic (RSO_2H) and sulfonic (RSO_3H) derivatives.

The oxidation of PSH by H_2O_2 via reaction 94 to yield the sulfenic acid derivative PSOH is repairable in that it can be reversed by addition of excess cysteine or other thiols.¹²⁹



Inactivation yields for papain and GAPDH in oxygenated solutions are exceptionally high. For example, with air saturated 3×10^{-5} M papain under γ -radiolysis $G(\text{inact})_{\text{O}_2} = 4.8$ of which $G = 3.5$ is repairable on treatment with excess cysteine.¹²⁷ Similar high yields are observed with GAPDH.¹²⁸ With papain which has a single SH group (which is essential for enzymatic activity) only ~ 10 percent of the available OH radicals are involved in reaction 86 to yield non repairable damage. The remainder are removed through reactions at other side-chain loci and at the protein main-chain. Most of the sulfur chemistry in these systems arises from reactions of O_2^- and H_2O_2 with PSH as formulated in reactions 93,94.

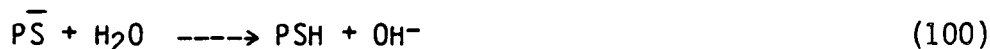
Similar studies have been made with lactate dehydrogenase.¹³⁰ With LDH the loss of enzymatic activity in oxygenated solution also involves SH oxidation at the active site. However, the inactivation yield with LDH is much lower with $G(\text{inact})_{\text{O}_2} = 0.12$. This low inactivation yield, as compared to papain and GAPDH, is attributed to the fact that the SH groups of papain and GAPDH are involved in substrate binding and are highly nucleophilic. Hence, they are more reactive towards O_2^- and H_2O_2 than the SH groups of LDH which do not have this function but conserve three dimensional structure through H bond formation.

The inactivation yield $G(\text{inact})_{O_2} = 0.12$ obtained with LDH falls within the range of inactivation yields observed with most other enzymes $G(\text{inact})_{O_2} = 0.1$ to ≈ 0.5 in oxygenated solution.^{131,132} There are two main reasons for the generally low values for enzyme inactivation by ionizing radiation: (1) Only a few enzymes such as papain and GAPDH have active sites reactive towards O_2^- and H_2O_2 (2) The attack of OH radicals is relatively non-specific and is widely distributed over many loci both main-chain and side-chain.

Major sites for reaction of e_{aq}^- with proteins in oxygen free solutions include disulfide linkages, peptide carbonyls and histidine side-chains. Electron trapping at disulfide bridges in proteins



yields the characteristic absorption band at ~ 400 nm observed with cystine and other simple disulfides.¹³³ The chemical consequences of e_{aq}^- attachment to S-S bonds of proteins are not fully understood. There appear to be two types of disulfide traps in proteins.^{108,112} In the one type, the PSSP adduct is short-lived and undergoes the dissociation



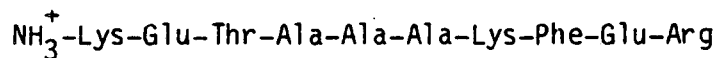
as is found with the simple disulfides, cystine etc. The production of PSH by the dissociation reaction 99,100 has been observed with a

number of aqueous proteins including papain¹³⁴ and Bowman-Birk proteinase inhibitor.¹³⁵ With papain, the dissociation reaction 99 contributes to the overall inactivation yield. The PSSP adducts of the second type are long lived and have the stability of -S-S-ions observed in aqueous glasses.^{125,136} Apparently the long lived PSSP adducts have structures and environments which prevent dissociation. Enzymes that show a high yield of long lived PSSP radicals with little or no short lived component are not significantly inactivated by e_{aq}^- .¹³³ It has been suggested that the long lived PSSP species are ultimately removed through back reaction with radicals formed through OH attack at various side chain and main-chain loci.¹²¹

The $\dot{P}S$ radicals formed in reaction 99 and in the OH reaction 92 can undergo combination reactions in O_2 -free solutions to yield higher molecular-weight dimer and trimer products which contribute to the combined aggregate yield. Radiation aggregates arise from both covalent and non-covalent binding. The latter aggregate forms dissociate to monomer in concentrated aqueous solutions of sodium dodecyl sulfate (SDS) or urea.⁸⁹ Recently, detailed studies have been made of the radiolytic formation of aggregate products in oxygen-free solutions of lactate dehydrogenase, LDH;¹¹⁰ ribonuclease, RNase;¹³⁷ bovine serum albumen, BSA.^{111,138} Gel filtration and gel electrophoresis techniques were employed in the separation of protein fractions in aqueous SDS solutions with and without added reducing agent, RSH.

The findings clearly show that two types of covalently bonded dimers are formed: those that contain disulfide crosslinks and those that contain other (non-reducible) covalent linkages. The combined yield of covalently linked dimers in these systems are in the range $G \sim 0.1$ to $G \sim 0.5$. Disulfide bridges appear to be less important (10 to 40 percent) than crosslinks derived from other types of radical-radical reactions. These other modes of radiation crosslinking are treated in following sections.

The S peptide of ribonuclease (RNase-S) has an interesting structure from the radiation chemical standpoint because it is made up of a single chain of 20 amino acids of known sequence with a single sulfur-containing amino acid residue (methionine)



The combination of S-peptide with S-protein is enzymatically active and the single methionine residue appears to be involved in the binding of S-peptide to S^c-protein.

Studies of the radicals formed in the reactions of photo-generated OH radicals ($\text{H}_2\text{O}_2 \xrightarrow{h\nu} 2\text{OH}$) with S-peptide (using t-nitrosobutane as the spin trap, (eg. 33) show the formation of both main-chain and side chain radicals.⁶⁵ The alanine main-chain and the methionine side-chain radicals were identified as major products. However, each of

these represented but one of a large number of radical products of each type. Earlier studies^{139,140} of amino acid destruction in the γ -radiolysis of S-peptide in oxygenated solution appear to be in general agreement with the spin-trap data. The observed amino acid losses expressed as changed residues/mole S-peptide (at 17 percent residual activity) correspond to

Met \sim 0.65, Ala \sim 0.25, Ser \sim 0.25, Phe \sim 0.2, Lys \sim 0.2,

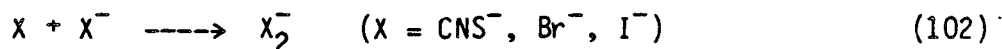
His \sim 0.18, Glu \sim 0.15, Arg \sim 0.13, Thr \sim 0.1, Asp \sim 0.1.

Column chromatography (on polyacrylamide gels) of the irradiated S-peptide (after mild hydrolysis at pH 3 for 30 minutes) showed no new peaks and the authors concluded that main chain fragmentation resulting from OH attack (eq. 86,87, etc.) does not occur. However, the single main peak showed broadening and a pronounced shift in the maximum to the lower-molecular weight side. This would be consistent with a more or less random fragmentation of the S-peptide main chain as a consequence of OH attack. A re-examination of this system using the more sensitive SDS-polyacrylamide gel electrophoresis technique would be interesting as would a keto acid analysis of the unidentified carbonyl product fraction.

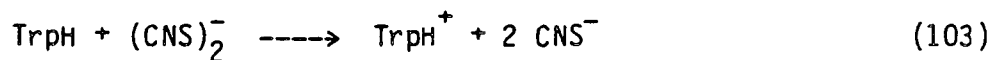
B. Aromatic-Unsaturated Residues

Most enzymes (with the exception of certain SH enzymes discussed in Sec. VA above) are inactivated by OH attack in oxygenated solution with relatively low efficiencies and have $G(\text{inact})_{O_2}$ values in the range 0.1 to 0.5.¹³¹ Although early pulse radiolysis and product analysis data showed that the aromatic-unsaturated residues are important loci

of OH attack in these systems, such studies provided only limited information on the amino acids essential to the activity of a particular enzyme.^{133,141} In N_2O saturated solution ($N_2O + e_{aq}^- \rightarrow N_2 + OH^-$), the transient spectra from the reaction of OH with most proteins (for example, pepsin,¹⁴² RNase,¹¹³ lysozyme¹⁴⁶ and α -chymotrypsin¹¹⁸) are relatively featureless and reflect the non-specificity of the OH reactions which are widely distributed over many sites both side-chain and main-chain. However, the finding that inorganic free radical ions generated via



are relatively unreactive towards aliphatic amino acids but show specificity in their reactions with aromatic and sulfur containing residues provided a new approach to the identification of residues crucial to the activity of a particular enzyme. In the early study¹⁴³ the specificity of the reaction of $(CNS)_2^-$ radicals with tryptophan



was used to show that radiation damage to tryptophan leads directly to the loss of lysozyme activity. Combined pulse radiolysis and enzyme inactivation studies of the reactions of $(CNS)_2^-$, Br_2^- and I_2^- radicals with numerous enzymes have led to the identification of amino acid residues essential to the enzymatic activity of: lysozyme [tryp], ribonuclease [his], chymotrypsin [his], trypsin [his];¹²⁰ pepsin [tryp],¹⁴² papain [cys], lactate dehydrogenase [cys].¹³⁰

Spin-transfer reactions between tryptophan radicals and tyrosine residues



in aqueous peptide and protein systems have been identified and studied kinetically.¹⁴⁴⁻⁶ Azide radicals generated pulse radiolytically via

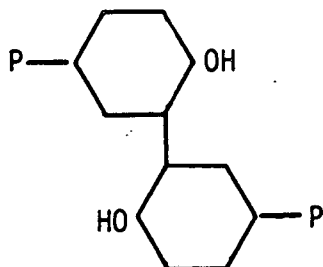


react selectively at tryptophan residues in neutral solution



The migration of damage from tryptophan to tyrosine through reaction 105 appears to proceed by direct H transfer rather than by charge conduction through the peptide chain or across hydrogen bonds. Measurements of the rates of reaction 105 are being used in studies of the effects of environment on protein structure and conformation.

The crosslinking of simple peptide derivatives of the unsaturated amino acids, phenylalanine, tyrosine, tryptophan and histidine in oxygen free solution has been discussed in Sec. III B. There now is definite evidence that similar processes are involved in the radiation induced dimerization of proteins. The di-tyrosyl crosslink



has been detected through of its strong characteristic fluorescence spectrum with $\lambda_{\text{max}} = 400 \text{ nm}$ as a product of the γ -radiolysis of aqueous insulin, ribonuclease, papain, collagen, histone, and

lysozyme.^{20,90,91,146} Although the observed yields of di-tyrosyl crosslinks in these systems, $G=0.1$, is quite low as compared to $G(OH)$, their detection suggests that similar crosslinking reactions at other sites are also involved but are less readily identifiable.²⁶

C. Aliphatic Residues

In the radiolysis of most aqueous enzymes the relative amino-acid contents of the protein are such that a major fraction of the OH radicals are removed at the more reactive sites, viz, the aromatic-unsaturated and the sulfur-containing residues. However, other important classes of protein have relatively higher aliphatic amino-acid contents which offer additional competing loci for OH attack. For example, studies of amino-acid loss in the γ -radiolysis of oxygenated solutions of heme α -globin,¹⁴⁷ soluble collagen¹⁴⁸ and thymus histone^{12,13} show that damage to aliphatic residues accounts for 75, 85 and 80 percent respectively of the observed chemical change initiated by OH attack. The aliphatic amino acids most affected in terms of the number of residues damaged include serine, threonine, proline, leucine, lysine and arginine.

The histone group of chromosomal proteins^{6,7} (H1,H2A,H2B,H3,H4) are closely related highly basic molecules characterized by (a) high contents of lysine and arginine (b) the absence of tryptophan and cysteine (with the exception of H3 which contains one cysteine residue) (c) a relatively low content of phenylalanine, tyrosine and histidine which for total thymus histone account for less than 6 mole percent of the amino acid content. Histone H1 has an aliphatic content of

~ 98 mole percent. In the studies of amino-acid destruction in the γ -radiolysis of thymus histone^{12,13} referred to above, the histones were prepared by dissociating the thymus deoxyribonucleohistone complex in 4M NaCl. The soluble histones were separated by centrifugation (70,000xg) and the supernatant was dialyzed against water until chloride free. The absorption spectrum of the final histone solution was recorded over the range 210 to 330 m μ to confirm the absence of measurable amounts of DNA. Prior to irradiation the histone solutions (0.007%) were saturated with oxygen and were re-oxygenated at 50 k-rad intervals. The observed G values for amino acid destruction in decreasing order correspond to:

lys ~0.31, leu ~0.16, arg ~0.15, tyr ~0.16, ser ~0.13, his ~0.13, val ~0.10, ile ~0.10, phe ~0.09.

In a parallel study, the deoxyribonucleohistone complex was irradiated under identical conditions. The total yield for amino acid destruction in this system was found to be essentially the same (within \pm 5%) as that obtained in the γ -radiolysis of the separated histones. The histone chromosomal-proteins act as efficient protective agents towards the DNA component. Significant destruction of purine and pyrimidine bases was not observed at dosages as high as 100 krad. Most of the OH-induced chemistry occurred at the histone moiety.

The significance of these findings in radiation biology remains to be established. It is to be noted that the amino acid sequences of the histones, particularly of histones H3, H4 and interior sequences of H1, are the most rigidly conserved of all proteins so far studied;⁶

It has been suggested that the remarkable resistance of histone sequences to change through the evolution of eukaryotic cells implies that each and every residue of the sequences is essential for the biological function of the deoxyribonucleohistone complex. During the past few years it has become increasingly clear that histones play key roles in determining both structural and transcriptional properties of the chromosome.⁵⁻⁷ For example, recent studies of the properties of active and repressed genes in somatic cell chromatin indicate that the repressed state of certain genes is directly maintained by histone H1 binding to specific DNA sites.¹⁴⁹⁻¹⁵⁰

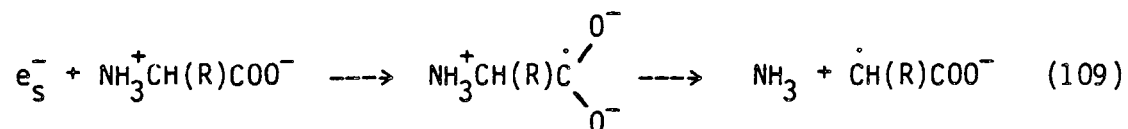
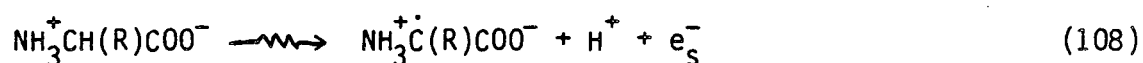
Hence, radiation damage to one or a few amino acid residues in the histone component could lead to a decrease in the stability of the DNA-histone complex which has been observed with a number of different experimental procedures.^{13,14,151,152} Oxidative cleavage of the histone main-chain by OH attack via reactions 84,85 would be a particularly destabilizing form of damage. Such radiation chemical damage could lead to the exposure of genetic information which is not required by the cell and which could lead to cell dysfunction. Detailed studies of the radiation chemistry of histones and other protein components of chromatin in oxygenated systems both in vitro and in vivo are indicated. Sensitive analytical procedures,¹⁵³ which were developed out of earlier studies of the radiolytic oxidation of protein in dilute aqueous solution,⁴⁶ could be useful in studies of histone oxidation both in-vitro and in-vivo at dosages in the kilo-rad range commonly used in biological studies. The analysis are based on the finding

that reactive carbonyl groups are major products of protein oxidation initiated by OH attack as discussed in Sec. II B, III B. The method is of particular interest in relation to the radiolytic oxidation of histones since these proteins have high contents of aliphatic amino-acids - particularly serine, threonine proline, lysine and arginine which characteristically yield carbonyl products from both main-chain and side-chain oxidation (Sec. III B).

The γ -irradiation of deoxynucleoprotein in oxygen-free systems leads to the formation of DNA-protein crosslinks which appear to be covalent in nature in that they are stable to salt and detergent treatment.^{16,17} Recent work indicates that the core histones H2A, H2B, H3, H4 are the specific proteins involved in the radiation crosslinking reaction in chromatin.¹⁸ Oxygen inhibits DNA histone crosslinking presumably by reacting preferentially with DNA and protein radicals.

VI. Solid State SystemsA. Amino Acids

The identification of the reductive deamination reaction 2 in aqueous amino acid systems (Sec. IIA) led to the proposal that dissociative electron capture is also involved in the formation of ammonia as a major product in the radiolysis of the α -amino acids in the solid state.¹⁵⁴ Ionic processes in these irradiated polar solids would then be represented by:



The subsequent reactions 4-7 of the aqueous mechanism occur in part in the solid and are completed on dissolution of the irradiated solid in O_2 -free water.

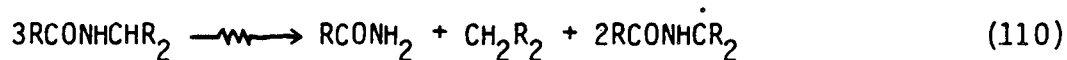
It was soon found that keto acids and fatty acids are indeed formed as major products in the γ -radiolysis of solid glycine and alanine with $G(\text{NH}_3) = G(\text{RCO}_2\text{COOH}) + G(\text{CH}_2\text{RCO}_2\text{COOH}) = 5$.^{155,156} Results obtained with ESR techniques provide physical evidence that on irradiation at 77°K the initially observed radical corresponds to the electron adduct $\text{NH}_3^+\text{CH}(\text{R})\overset{\cdot}{\text{C}}\text{OO}^-$ which dissociates on warming to yield $\text{NH}_3 + \dot{\text{C}}\text{H}(\text{R})\text{COO}^-$.^{157,158} The similarities between the radiation chemistry of these simpler amino acids in the solid state and in aqueous solution are quite striking.

Reaction 108 represents a major path for removal of e_s in the radiolysis of most α -amino acids in the solid state as evidenced by both product analysis^{32,156} and ESR studies¹⁵⁹⁻¹⁶² of various aliphatic, aromatic, and sulfur-containing derivatives.

B. Peptides and Polypeptides

Radiolytic cleavage of the peptide N-C bond in solid-state systems with formation of amide, fatty acid and keto acid functions as major products was first identified in radiation chemical studies of the N-acyl and polyamino acid derivatives of alanine.^{67,163,164} Detailed chemical separations of products formed in the radiolysis of polyalanine and N-acetylanine give $G(\text{amide}) \approx 3$, $G(\text{propionic acid}) \approx 2$, $G(\text{pyruvic acid}) \approx 1$, $G(\text{dimer}) \approx 2$. The irradiated solids were dissolved in O_2 -free water and analyzed using the techniques of liquid-phase (partition) chromatography, gas-phase chromatography and mass spectrometry. The authors of a recent study¹⁶⁵ in which irradiated solid N-acetylanine was simply heated to 70-80°C in the injection port of a gas chromatograph have reported propionic acid and acetamide to be only minor products with G values of ~ 0.1 and ~ 0.2 respectively; apparently product recovery by this procedure is far from quantitative.

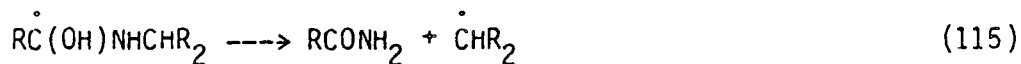
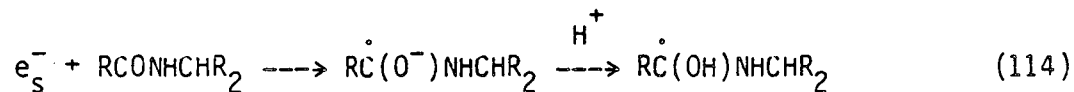
The major radiation-induced chemistry leading to the observed product yields were correlated in terms of the reaction stoichiometries^{163,164}



where RCONHCR_2 represents the long-lived radical products observed in the early ESR studies of γ -irradiated solid peptides.¹⁶⁶ Dimerization of these radical products occurs on dissolution of the irradiated solid in O_2 -free water. The dehydropeptides formed in the radiation induced step 111 are labile and undergo hydrolysis to give keto acid and additional amide.



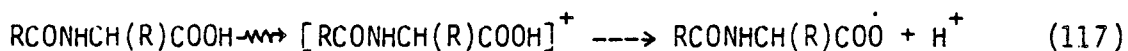
ESR studies have since shown that the formation of amide and fatty acid as via the stoichiometry of equation 110 involves the intermediate ionic processes:^{162,167-169}



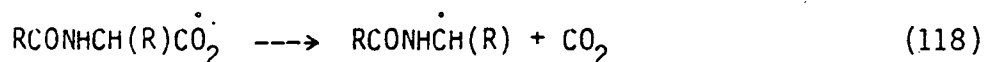
to give the observed over-all product stoichiometry.

Other ESR studies,¹⁶⁹ of a large number of γ -irradiated solid peptides have shown that the long-lived (final) radicals for essentially all aliphatic residues including methionine corresponds to the α -carbon radical RCONHCR_2 as shown in eq. 116. With tyrosine and cysteine peptides the long-lived radicals are at side-chain loci, viz $\dot{\Phi}\text{O}$ and RS respectively.^{169,167}

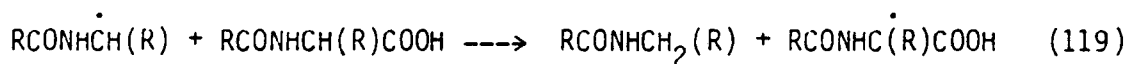
The radiation chemistry of the lower-molecular weight peptides such as the N-acetyl amino acids and the di and tripeptides is somewhat more complicated than that given in the scheme of reactions 113-116. In these systems, the carboxyl group represents the major locus of positive-hole formation i.e.,



The ionization step 117 is then followed by



and the abstraction reaction

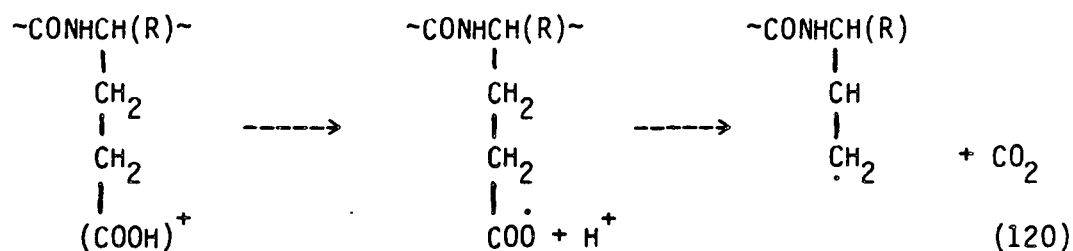


to yield the long-lived α -carbon radical¹⁶⁹ plus carbon dioxide and the N-acylamide as additional final products.^{165,170} Both the decarboxylated radical, RCONH $\dot{\text{C}}\text{HR}$, and the α -carbon radical, RCONHC $\dot{\text{C}}\text{(R)COOH}$ have been spin-trapped on dissolution of γ -irradiated N-acetyl amino acids and dipeptides in aqueous solution of t-nitrosobutane as the trapping reagent.¹⁵⁹

Cleavage of the peptide main-chain with formation of amide and fatty acid functions is a major process in the solid-state radiolysis of peptide derivatives of almost all aliphatic amino acids. Values of G(amide) ~ 3 and G(fatty acid) ~ 2 have been obtained with peptide

derivatives of glycine, alanine, α -aminobutyric acid, glutamic acid, leucine, and methionine.^{32,164} The α -carbon radical, $\sim\text{CONH}\dot{\text{C}}(\text{R})\sim$, is the long-lived radical species in each case as observed by direct ESR measurements of the irradiated solids.¹⁶⁹

With peptide derivatives of aspartic and glutamic acids, the positive hole is located preferentially at the side-chain carboxyl group.



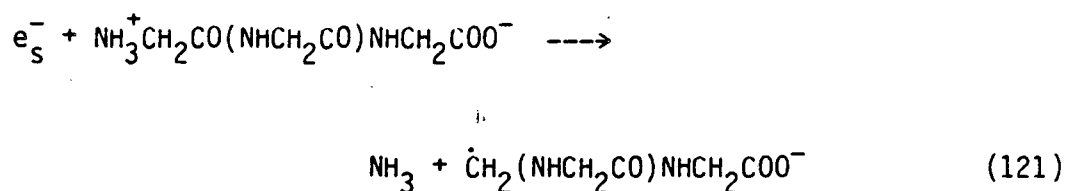
where the radical product of reaction 120 then abstracts H from the main-chain to form the α -aminobutyric acid derivative as a major product.^{169,171,172}

Although both methionine and cysteine undergo C-S bond cleavage on reaction with the hydrated electron, e_{aq}^- , in aqueous solution (reactions 78, 82, 83) the evidence from both product analysis¹⁵⁶ and ESR^{167,169} studies is that such reactions are relatively unimportant in the solid state. The C=O linkage of the peptide bond appears to be the major trapping center for e^- in the radiolysis of methionine and cysteine peptides as solids.

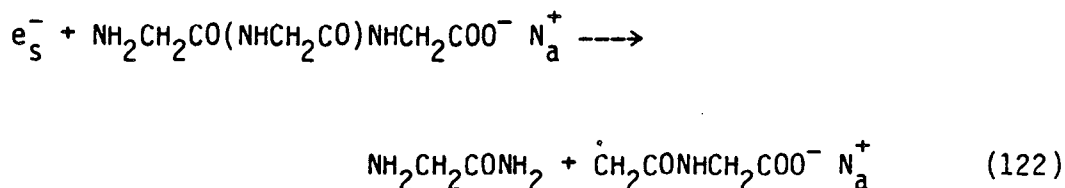
The yield of main-chain cleavage as measured in terms of amide production is appreciably lower with peptide derivatives of the aromatic amino acids, phenylalanine and tyrosine.¹⁶⁴ The ESR evidence

is that the unsaturated side chains of phenylalanine, tyrosine, and histidine compete effectively with the peptide bond for e_s^- in the solid state.¹⁶⁹

Although the reductive N-C cleavage reactions 113,114 represent a major path for removal of e_s^- in the γ -radiolysis of N-acetylamino acids and polyamino acids in the solid state, this is not the case with the oligopeptides, i.e. with the di, tri and tetrapeptide derivatives in their zwitterion forms. In these systems the C=O bond of the positively charged N-terminal residue is the major trapping center for e_s^- . In the γ -radiolysis of solid triglycine zwitterion, for example, the predominant mode for e_s^- removal leads to the deamination reaction:

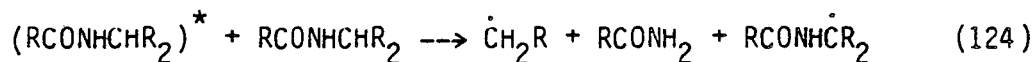
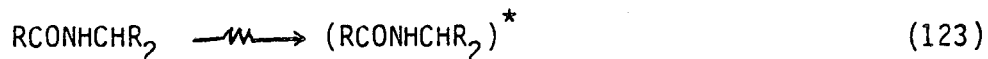


However, with triglycine in the basic form the deamination reaction



occurs preferentially.¹⁷³

Studies of the effects of added second-solutes on the radiation chemistry of acetylalanine in aqueous glasses and in concentrated aqueous solutions suggest that excited states may also be involved in the radiolytic cleavage of the peptide chain¹⁷⁴ i.e.



followed by the abstraction reaction 116 above. This reaction sequence gives the same product stoichiometry as the ionic mechanism give in equations 113-116.

C. Proteins

Studies of amino acid destruction in irradiated solid proteins¹⁷⁵⁻¹⁷⁹ are of limited value in elucidating reaction mechanisms since doses of 50 to 100 Mrad may be required to obtain a reasonably accurate measurement of the percent decrease of a particular amino acid. At these dose levels there is extensive denaturation; for most enzymes, the G value for inactivation is in the order of unity i.e. only ~ 100 eV absorbed energy is required to inactivate an individual enzyme macromolecule.^{180,181} Amino acid loss studies have, however, been useful in showing that there is no highly preferential destruction of a relatively few amino acids in irradiated solid proteins. Variations in the "radiation sensitivity" of the various amino acids range over a factor of ~ 5.

The formation of decomposition products can usually be followed at lower dosages because of the generally lower background values. Yields of major decomposition products formed in the γ -radiolysis of a number of globular and fibrous proteins in the absence of oxygen include: amide function with $G \sim 2.5$,^{175-179,182} carbonyl (keto acid plus alde-

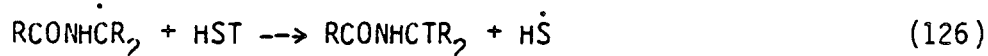
hyde) with $G \sim 1$,^{175,176,182} fatty acids with $G \sim 1.5$ ¹⁸³ and long-lived radicals with $G \sim 3.5$.^{184,185} These data were obtained in radiation chemical studies of serum albumen,¹⁷⁵ α -chymotrypsinogen,¹⁸³ lysozyme¹⁸² trypsin,¹⁷⁷ collagen^{176,178} and gelatin.⁴⁶ The fatty acid yield of $G \sim 1.5$ given above corresponds to the G value for acetic and propionic acid production in γ irradiated α -chymotrypsinogen calculated in terms of the fraction of energy absorbed directly in glycine and alanine residues. These composite data taken together strongly support the hypothesis that the intermediate processes discussed in equations for simple peptides and polypeptides also have validity in the radiolysis of solid proteins.

Most of the proteins listed above, when irradiated in vacuo at room temperature, give an ESR signal which is predominantly a well resolved doublet of about 20 gauss spacing characteristic of carbon-centered radicals^{186,187} This protein doublet appears to be a composite since 15 of the 20 common amino acid residues yield long-lived $RCONHCR_2$ radicals which exhibit doublet ESR signals with a ~ 20 gauss spacing.¹⁶⁹ With many proteins irradiated at room temperature, a second ESR signal characteristic of cystine is observed in lower yield.^{186,187} It has been suggested¹⁸⁸ that electron capture via



occurs in competition with the addition of e_s^- to the peptide carbonyl via reaction 114.

Distribution of the long-lived carbon-radical centers among the various amino acid residues in irradiated proteins has been determined by exposing them to tritium labelled hydrogen sulfide (HST) and hydrogen iodide (TI) which react essentially quantitatively over a period of a few hours to yield tritium labelled amino acids via¹⁹⁰

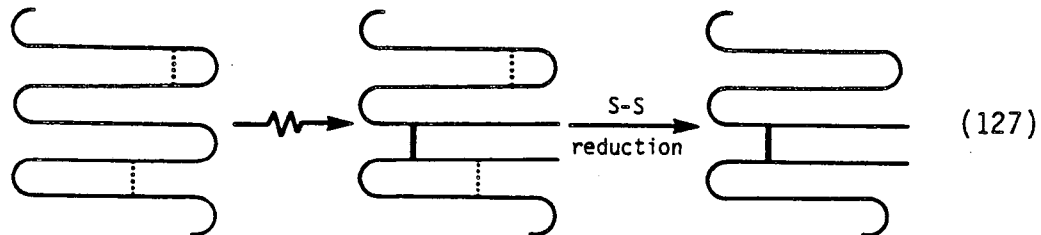


After exposure to HST, the proteins were dissolved in water and allowed to stand at room temperature to remove exchangeable tritium. The proteins were then lyophilized, hydrolysed and assayed using an amino acid analyzer in tandem with a scintillation flow counting system. A significant finding is that the long-lived radiation-induced radical centers are distributed amongst all of the various amino acid residues in the protein. In most proteins, proline, methionine, lysine, histidine, threonine and serine show a higher activity than that shown by other amino acids.

The formation of amide and fatty acid as major products in the radiolysis of solid polypeptides and proteins explicitly states on the basis of reactions 110-116 that cleavage of the peptide main-chain occurs in all of these systems. Such cleavage has been directly observed with polyamino acids and with the fibrous proteins collagen and gelatin.^{185,191} Radiolysis of these solids in the absence of oxygen produces main-chain breaks with $G \sim 1$ to 2 as evidenced by the observed decrease of the average molecular weight of the irradiated samples. Supporting

evidence that such breaks occur through reductive cleavage by e_s via reaction has been obtained in studies of the effects of metal ions (Cu^{2+} , Fe^{2+} , Ni^{2+}) on radiation-induced molecular weight changes in solid collagen.¹⁹² It was found that the presence of cupric ion at a ratio of 1 Cu^{2+} /10 amino acid residues, completely blocks the formation of main-chain breaks in solid collagen irradiated with a dose of 25 Mrad. Removal of e_s via $\text{Cu}^{2+} + e_s \rightarrow \text{Cu}^{1+}$ would be expected to eliminate any contribution of main-chain cleavage through reaction.

Although main-chain breaks are observed with fibrous proteins, studies of the effect of γ -rays on globular proteins (e.g. ribonuclease, lysozyme, creatine phosphotransferase) in the evacuated solid state consistently show only small amounts of main-chain fragmentation even after reduction of intramolecular disulfide bonds.^{182,185,191} Yet, as we have noted above, the observed amide and fatty acid yields for globular and fibrous proteins are very similar. Now, in most of these comparative studies, the solid proteins were irradiated in vacuo and then dissolved in air-saturated water for analysis. On dissolution of the irradiated globular proteins, crosslinking of the long-lived carbon-centered radicals would be favored because of the constraints imposed by the secondary and tertiary structure. Oxygen penetration into the globule would be relatively slow. With polyamino acids and fibrous proteins, such constraints are minimal and the dissolution of the radical fragments and their scavenging by dissolved oxygen, via reaction, would be favored. Main-chain breaks in the globule would be masked by the formation of the new intramolecular crosslinks i.e.



where --- represent disulfide bonds of the native protein. This formation is in accord with the stoichiometric requirements of reactions 110-116 - i.e. each (amide) main-chain break is accompanied by formation of a pair of carbon centered radicals. The radiation-induced crosslinks shown in equation 127 would be formed through various combination reactions involving α -carbon radicals, $RCONH\dot{C}R_2$, and the side-chain radicals e.g. $\dot{\phi}O$ and $R\dot{S}$ discussed above. All such combination reactions yield α, α' -diaminodicarboxylic acid derivatives.²⁶ If the globular protein is irradiated in an air atmosphere, then a greater fraction of the long-lived radicals would be scavenged by oxygen via reaction 15, 15a resulting in an enhancement in the main-chain degradation yield via reactions 16-24. Such an enhancement has been observed with ribonuclease.¹⁹³

It is of interest to note that a corollary of this model for radiation damage summarized in equation 127 is that the product protein species should, because of the masked main-chain break, exhibit a sequential amino-acid analysis pattern different from that of the unirradiated material. Such an analysis could also provide information on the precise locus of both the break and the crosslink.

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ACKNOWLEDGMENT

This work was supported by the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

This report was done with support from the Department of Energy. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the Department of Energy.

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