Reaction Mechanisms in the Radiolysis of Peptides, Polypeptides, and Proteins†

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I. Introduction

Detailed knowledge 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]

Warren M. Garrison was born in Seattle, WA, and obtained his B.S. and M.S. degrees at the University of California, Berkeley. He received his Ph.D. degree at New York University in 1942. In that same year he joined the Manhattan Project and served first at the Metallurgical Laboratory, University of Chicago, and later at the Hanford Engineering Works, Hanford, WA. In 1947 he joined the staff at the Lawrence Berkeley Laboratory where he is now Staff Senior Scientist, Emeritus. His research interests are centered on the radiation chemistry of biochemical systems, particularly amino acids, peptides, and proteins, and its applications in radiation biology and in the radiation sterilization of foods and drugs.

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 covalent DNA–protein cross-links 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 cross-links in γ-irradiated chromatin.[18,19] Radiation-induced cross-linking of histone octamer complexes has

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recently been reported;[20] these complexes consist of two sets of core histones and are major components of the nucleosome, a basic subunit of chromatin.[21,22]

It is now well established that certain processes of aerobic cell metabolism involve the enzymatic production of the superoxide) radical O_2^- and its subsequent removal by superoxide dismutase to yield hydrogen peroxide and oxygen: $2O_2^- + 2H_2O \rightarrow H_2O_2 + 2OH^- + O_2$.[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'-diamino dicarboxylic 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'-diamino dicarboxylic 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]

$$H_2O \rightsquigarrow H_2O_2,\ H_2,\ OH,\ H,\ e_{aq}^-,\ H^+ \qquad (1)$$

where e_{aq}^- represents the hydrated electron.[30] For γ rays and fast electrons the yields of the radical products correspond to $G(OH) = 2.8$, $G(e_{aq}^-) \simeq 2.7$, $G(H) \simeq 0.55$ 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.[31,32] 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[32]

$$e_{aq}^- + {}^+NH_3CH(R)COO^- \rightarrow NH_3 + \dot{C}H(R)COO^- \qquad (2)$$

$$OH + {}^+NH_3CH(R)COO^- \rightarrow H_2O + {}^+NH_3\dot{C}(R)COO^- \qquad (3)$$

Subsequent reactions include

$$\dot{C}H(R)COO^- + {}^+NH_3CH(R)COO^- \rightarrow CH_2(R)COO^- + {}^+NH_3\dot{C}(R)COO^- \qquad (4)$$

$$\dot{C}H(R)COO^- + {}^+NH_3\dot{C}(R)COO^- \rightarrow CH_2(R)COO^- + {}^+NH_2{=}C(R)COO^- \qquad (5)$$

$$2{}^+NH_3\dot{C}(R)COO^- \rightarrow {}^+NH_2{=}C(R)COO^- + {}^+NH_3CH(R)COO^- \qquad (6)$$

A small fraction of the ${}^+NH_3\dot{C}(R)COO^-$ radicals undergo dimerization to yield α,α'-diaminosuccinic acid. The labile imino acid derivative produced in the disproportionation steps 5 and 6 hydrolyzes spontaneously

$$H_2O + {}^+NH_2{=}C(R)COO^- \rightarrow {}^+NH_4 + RCOCOO^- \qquad (7)$$

The overall stoichiometry of reactions 2–7 gives

$$G(NH_3) \simeq G(RCOCOOH) + G(CH_2RCOOH) \simeq 5$$

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

$$e_{aq}^- + {}^+NH_3CH(R)COO^- \longrightarrow {}^+NH_3CH(R)\dot{C}\begin{matrix}O^-\\O^-\end{matrix} \qquad (2a)$$

and that the radical anion intermediate then dissociates

$${}^+NH_3CH(R)\dot{C}\begin{matrix}O^-\\O^-\end{matrix} \longrightarrow NH_3 + \dot{C}H(R)COO^- \qquad (2b)$$

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, $\dot{C}H(R)COO^-$, 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} s) of ionizing radiation and the transient free radicals are monitored and identified by absorption spectroscopy.[35] Electron spin resonance (ESR) spectroscopy[36] 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 77 K which then dissociated to give the fatty acid radical $\dot{C}H(R)COO^-$ (reaction 2b) on warming to 180 K.

With the aliphatic α-amino acids of higher molecular weight, i.e., with α-aminobutyric, 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 function.[42] Detailed reaction mechanisms involved in the radiolysis of the various amino acid side chains are treated in detail in section 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

$$e_{aq}^- + O_2 \rightarrow O_2^- \quad (8)$$

$$O_2^- + H_2O \rightleftarrows HO_2 + OH^- \quad (9)$$

$$2HO_2 \rightarrow H_2O_2 + O_2 \quad (10)$$

where $pK_9 \simeq 4.7$.[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

$$O_2 + {}^+NH_3\dot{C}(R)COO \rightarrow {}^+NH_2{=}C(R)COO^- + HO_2 \quad (11)$$

which hydrolyses spontaneously to give ammonia and keto acid:

$$H_2O + {}^+NH_2{=}C(R)COO^- \rightarrow {}^+NH_4 + RCOCOO^- \quad (12)$$

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 $G(NH_3) \simeq G(\text{Carbonyl}) \simeq G(OH) \simeq 3$.[32] The oxidative deamination yield decreases with increasing length of the aliphatic side chain. With norleucine, $^+NH_3CH(CH_2CH_2CH_2CH_3)COO^-$, only a small fraction of the OH radicals are removed through H abstraction at the α-carbon position to give $G(NH_3) \simeq 0.3$.

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]

$$RCONHCHR_2 + O_2 + H_2O \rightarrow RCONH_2 + R_2CO + H_2O_2 \quad (13)$$

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

$$OH + RCONHCHR_2 \rightarrow H_2O + RCONH\dot{C}R_2 \quad (14)$$

$$O_2 + RCONH\dot{C}R_2 \rightarrow RCONHC(\dot{O}_2)R_2 \quad (15)$$

$$\rightarrow RCON{=}C(R)_2 + HO_2 \quad (15a)$$

The subsequent steps were written

$$HO_2 + RCONHC(\dot{O}_2)R_2 \rightarrow RCONHC(OOH)R_2 + O_2 \quad (16)$$

$$H_2O + RCONHC(OOH)R_2 \rightarrow RCONHC(OH)R_2 + H_2O_2 \quad (17)$$

$$RCONHC(OH)R_2 \rightleftarrows RCON{=}CR_2 + H_2O \quad (17a)$$

$$H_2O + RCONHC(OH)R_2 \rightarrow RCOOH + NH_3 + R_2CO \quad (18)$$

Much of the subsequent information on the detailed mechanisms of oxidative main-chain degradation has been derived from studies involving 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 $G(NH_3) \simeq G(R_2CO) \simeq G(OH) \simeq 3$. Quantitative assays of the ammonia and carbonyl yields from a series of model peptide derivatives, including acetylglycine, acetylalanine, glycine anhydride, etc., consistently show $G(NH_3) \simeq 3$ 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 $G(R_2CO) \simeq 1$. 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 unsepcified complexity in the chemistry of removal of the peroxy radicals $RCONHC(\dot{O}_2)R_2$.

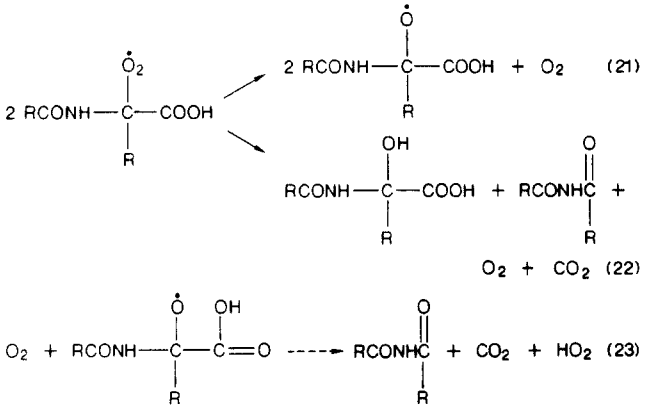
To obtain specific information on these questions, the radiolysis was carried out using ferric ion instead of O_2 as the scavenger of intermediate radicals formed by OH attack on *N*-acetylglycine and *N*-acetylalanine.[48] Heavy metal ions Fe^{3+} and Cu^{2+} oxidize organic free radicals in aqueous solution and such reactions in the case of the peptide radical $RCONH\dot{C}(R_2)$ would correspond to

$$Fe^{3+}(H_2O) + RCONH\dot{C}R_2 \rightarrow RCON{=}CR_2 + Fe^{2+} + H^+ + H_2O \quad (19)$$

$$Fe^{3+}(H_2O) + RCONH\dot{C}R_2 \rightarrow RCONHC(OH)R_2 + Fe^{2+} + H^+ \quad (20)$$

The oxidation products of reactions 19 and 20 are identical with the postulated products of reactions 16–18 and would then yield amide and carbonyl on hydrolysis. It was found that the γ-radiolysis of 0.1 M *N*-acetylglycine and *N*-acetylalanine in O_2-free solution containing 0.05 M Fe^{3+} gives the product stoichiometry $-G(\text{peptide}) \simeq G(NH_3) \simeq G(\text{RCOCOOH}) \simeq 3.2 \simeq G(OH) + G(H_2O_2)$. 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 $RCONHC(O_2)R_2$ with HO_2 via step 16 occurs in competition with



In the case of *N*-acetylalanine the diacetamide product, RCONHCOR, of reactions 22 and 23 is hydrolytically labile, and under mild differential hydrolysis in dilute base is converted to acetamide and acetic acid.

$$H_2O + \text{RCONHCOR} \rightarrow \text{RCONH}_2 + \text{RCOOH} \quad (24)$$

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

$$G(CH_3COCOOH + CH_3CHO) \simeq 1$$
$$G(CH_3COOH) \simeq 2.5 \qquad G(CO_2) \simeq 2$$

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:

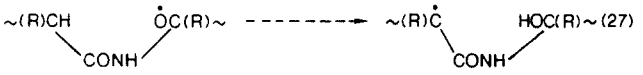
$$OH + {}^+NH_3CH(R)CONHCH(R)COO^- \rightarrow H_2O + {}^+NH_3CH(R)CONHC(R)COO^- \quad (25)$$

The chemical evidence is that ~90% 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 ~CONHC(R)~ 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 $k_{14} \sim 10^9$ M^{-1} s^{-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 1.2×10^9 M^{-1} s^{-1} and 1.8×10^9 M^{-1} s^{-1}, respectively.

In the presence of oxygen

$$O_2 + {}^+NH_3CH(R)CONHC(R)COO^- \rightarrow {}^+NH_3CH(R)CONHC(O_2)(R)COOH \quad (26)$$

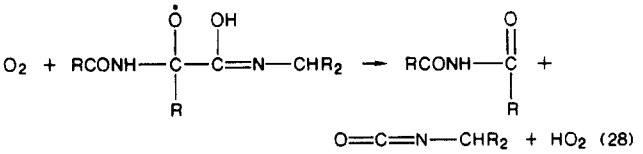
The subsequent chemistry is, in part, quite analogous to that observed in the radiolytic oxidation of *N*-acetylamino acids via the sequence 14–18, 21–23 to yield amide and the lower fatty acid. However, with the oligopeptides, the amide yield approaches $G(NH_3) \simeq 5$ which is considerably higher than the value $G(NH_3) \simeq 3 \simeq G$(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 $G(NH_3) \simeq 4.8$, G(HCOOH) $\simeq 1.7$, G(CHOCOOH) $\simeq 1.9$.[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 $G(>CO) \simeq 2$ with $G(NH_3) \simeq 4.8$.[52]

Product yields in the γ-radiolysis of polyalanine are consistent with the reaction sequence formulated above, i.e., $G(NH_3) \simeq 4.0$, G(RCOCOOH) $\simeq 1.2$, G(RCOOH) $\simeq 3.0$, and $G(CO_2) \simeq 2.4$.[47] With polyalanine at molecular weight ≃3000 it must be assumed that alkoxy radical formation via reactions 14, 15, and 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

$$H_2O + O{=}C{=}N{-}CHR_2 \rightarrow CO_2 + NH_2CHR_2 \quad (29)$$

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 RĊ(OH)NHCHR$_2$ radicals formed in reaction 30 has been

$$e_{aq}^- + \text{RCONHCHR}_2 \longrightarrow \text{R}\overset{\overset{O^-}{|}}{\dot{C}}\text{NHCHR}_2 \xrightarrow{H_2O} \text{R}\overset{\overset{OH}{|}}{\dot{C}}\text{NHCHR}_2 \quad (30)$$

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 RĊ(OH)NHCHR$_2$ radicals involves the back reaction (reconstitution)[57]

$$\text{RĊ(OH)NHCHR}_2 + \text{RCONHĊR}_2 \rightarrow 2\text{RCONHCHR}_2 \quad (31)$$

where RCONHĊR$_2$ corresponds to the product of OH attack via reaction 14. It was concluded that main chain cleavage via dissociative deamidation

$$\text{RĊ(OH)NHCHR}_2 \rightarrow \text{RCONH}_2 + \dot{C}\text{HR}_2 \quad (32)$$

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 32.[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 2-methyl-2-nitrosopropane

$$\dot{C}\text{HR}_2 + t\text{-Bu—N=O} \longrightarrow t\text{-Bu—}\overset{\overset{CHR_2}{|}}{N}\text{—O}^{\bullet} \quad (33)$$

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" RĊ(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]

$$e_{aq}^- + {}^+\text{NH}_3\text{CH(R)CONHCR}_2 \rightarrow {}^+\text{NH}_3\text{CH(R)Ċ(OH)NHCR}_2 + \text{OH}^- \quad (34)$$

which leads to deamination via

$${}^+\text{NH}_3\text{CH(R)Ċ(OH)NHCR}_2 \rightarrow {}^+\text{NH}_4 + \dot{C}\text{H(R)CONHCHR}_2 \quad (35)$$

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

$${}^+\text{NH}_3\text{CH(R)CONHCHR}_2 + \dot{C}\text{H(R)CONHCHR}_2 \rightarrow {}^+\text{NH}_3\text{CH(R)CONHĊR}_2 + \text{CH}_2\text{(R)CONHCH(R}_2\text{)} \quad (36)$$

and the dimerization reaction:

$$2\,{}^+\text{NH}_3\text{CH(R)CONHĊR}_2 \longrightarrow {}^+\text{NH}_3\text{CH(R)CONH}\overset{\overset{{}^+\text{NH}_3\text{CH(R)CONHCR}_2}{|}}{\dot{C}}\text{R}_2 \quad (37)$$

to yield the α,α'-diaminosuccinic acid derivatives.[26,63,64]

The yields of the reductive deamination reaction 34 and 35 with di and triglycine corresponds to $G(\text{NH}_3) \simeq 3 \simeq e_{aq}^-$. The free ammonia yield from tetraglycine is somewhat lower, $G(\text{NH}_3) \simeq 2.4$. With polyalanine the free ammonia yield decreases to $G(\text{NH}_3) \simeq 0.3$: This decrease in free ammonia is accompanied by an increase in "amide" cleavage resulting from capture of e_{aq}^- by C=O bonds away from the terminal residue via reactions 30, 32.[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 $k_{30} \sim 1 \times 10^9$ M^{-1} s^{-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 reactions.[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 (section 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

$$>\text{(H)CH} + \text{OH} \rightarrow >\text{(H)C}\bullet + \text{H}_2\text{O} \quad (38)$$

$$>\text{(H)C}\bullet + \text{O}_2 \rightarrow >\text{(H)C}\dot{\text{O}}_2 \quad (39)$$

followed by the characteristic reactions of alkyl peroxy radicals.[48–51]

$$>\text{(H)C}\dot{\text{O}}_2 + \text{HO}_2 \xrightarrow{H_2O} >\text{(H)COH} + \text{H}_2\text{O}_2 + \text{O}_2 \quad (40)$$

$$>\text{(H)C}\dot{\text{O}}_2 + >\text{(H)C}\dot{\text{O}}_2 \rightarrow >\text{(H)COH} + >\text{C=O} + \text{O}_2 \quad (41)$$

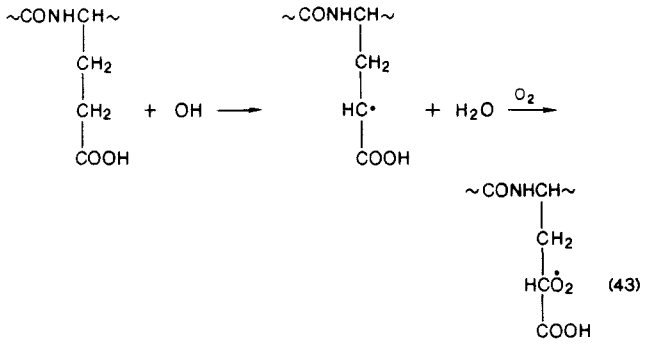
$$>\text{(H)C}\dot{\text{O}}_2 + >\text{(H)C}\dot{\text{O}}_2 \rightarrow 2>\text{(H)C}\dot{\text{O}} + \text{O}_2 \quad (41a)$$

$$>\text{(H)C}\dot{\text{O}} + \text{O}_2 \rightarrow >\text{C=O} + \text{HO}_2 \quad (42)$$

Oxidation can occur at any C–H position along the chain. Valine and leucine yield 3-hydroxyvaline and 3-hydroxy- plus 4-hydroxyleucine respectively as major products. Detailed chemical identifications and quantitative determinations of the various hydroxy and

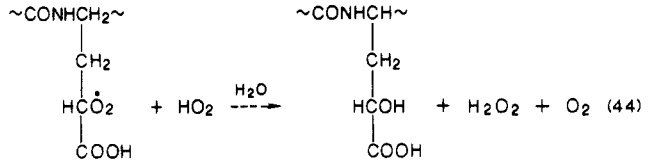
carbonyl products formed through OH attack at side chain loci of α-aminobutyric, valine, and leucine have been made.[66]

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 analysis[68] and spin-trapping methods.[65] With *N*-acetylglutamic acid in oxygenated solution, main-chain degradation via reactions 14–18 to yield amide and α-ketoglutaric acid functions accounts for ~30 percent of the OH radicals.[68] The remainder attack at the side chain via



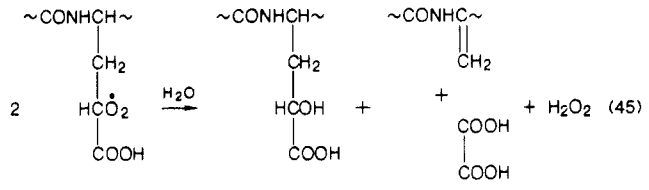
(43)

Part of the subsequent chemistry ($RO_2 + HO_2 \rightarrow$) is similar to that formulated in eq 40, i.e.,



(44)

However, the analogues of the competing reactions ($2RO_2 \rightarrow$) takes a more complicated form than that shown in eq 41 and 42, i.e.,



(45)

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.

$$\sim CONHC(=CH_2)CO\sim \rightleftarrows \sim CON=C(CH_3)CO\sim \quad (46)$$

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

$$\sim CON=C(CH_3)CO\sim + H_2O \rightarrow \sim CONH_2 + CH_3COCO\sim \quad (47)$$

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-saturated) are essentially independent of pH over the range pH 3 to 8 with G(amide) ≃ 2.3, G(α-ketoglutaric) ≃ 0.8, and G(pyruvic) ≃ 0.9. Similar product yields are obtained with polyglutamic acid solutions (0.15%, O_2-saturated) 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-ketoglutaric yield remains essentially constant over the entire pH range 8 to 3.

In interpreting these pronounced pH effects in the polyglutamic (PGA) 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% 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 → 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 RO_2 are frozen in a mixed spatial arrangement, and it is obvious that the probability of reaction 45 ($2RO_2 \rightarrow$) is greatly reduced, reaction 44 ($RO_2 + HO_2 \rightarrow$) 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 eq 11 and 12 above, e.g.,

$$\sim CH(R)OH + OH \xrightarrow{O_2} \sim CO(R) + H_2O + HO_2 \quad (48)$$

$$\sim CH_2NH_2 + OH \xrightarrow{O_2} \sim CHO + NH_3 + HO_2 \quad (49)$$

$$\sim CH_2NH(C=NH)NH_2 + OH \xrightarrow{O_2} \sim CHO + NH_2(C=NH)NH_2 + HO_2 \quad (50)$$

plus products of higher oxidation.

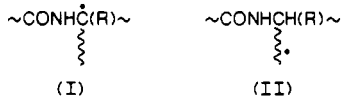
The reactions of OH radicals with peptide derivatives of the aliphatic amino acids outlined above, all involve the formation of carbon-centered radicals through H abstraction. Such reactions are of intermediate velocity with k values[60,69] in the range $1–5 \times 10^9$ M^{-1} s^{-1}.

2. Evacuated Solutions

The available data from product analysis,[67] pulse radiolysis,[55,60] and spin-trapping studies[62] in irradiated aqueous solutions and from ESR studies[61] 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 and glutamic, and their respective amides, asparagine and glutamine, (c) the basic

amino acids, lysine and arginine, and (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 M^{-1} s^{-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

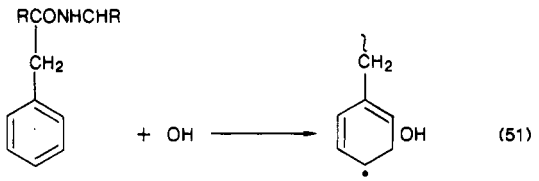


I radicals from glycine and alanine residues to give α,α'-diaminosuccinic and α,α'-diaminodimethylsuccinic acids respectively has been discussed. Higher molecular weight dimers such as α,α'-diaminopimelic and α,α'-diaminosuberic formed through dimerization of type II radicals have also been identified.[66] The formation of unsaturated dimers through combination of side-chain radicals derived from aromatic unsaturated amino acid residues is discussed below.

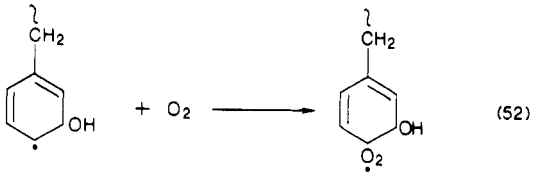
B. Aromatic Unsaturated Residues

1. Oxygenated Solutions

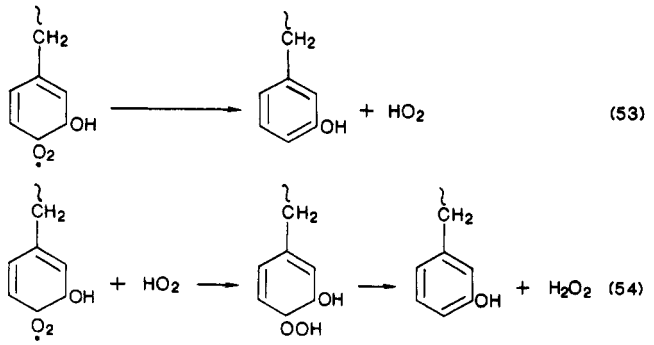
Reactions of OH radicals with the phenylalanine residue include: (1) H-abstraction at the main chain via reaction 14 to give the main-chain radical,[65] RCONHĊ(Φ)R, and (2) addition to the aromatic side chain[71,72] where reaction 51



represents the principal path for OH removal. In the presence of O_2, the main-chain radicals, RCONHĊ(Φ)R, undergo oxidative degradation with formation of amide, phenylpyruvic acid, and products of higher oxidation through reactions analogous to those formulated in eq 14–23. The hydroxycyclohexadienyl radicals formed through the OH addition reaction 51 react with O_2 to yield peroxy radical intermediates



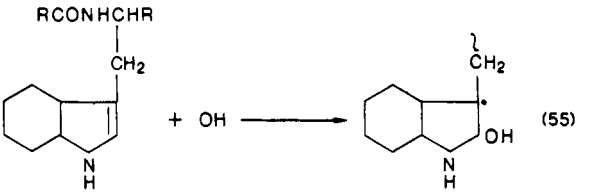
which undergo the subsequent reactions



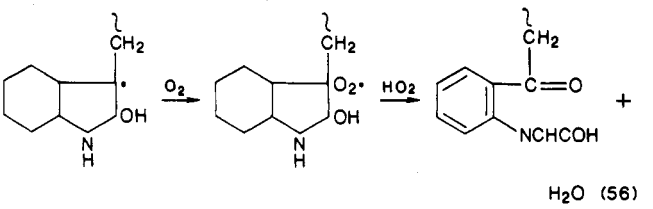
to yield tyrosine (ortho, meta, and para) as the major products.[71-73] In the radiolysis of aqueous benzene a fraction of the peroxy radicals formed in reaction 52 undergo rearrangement and further oxidation to yield β-hydroxymucondialdehyde.[73]

The radiolytic oxidation of tyrosine residues in O_2 saturated solution appears to involve reactions analogous to those given in eq 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.,

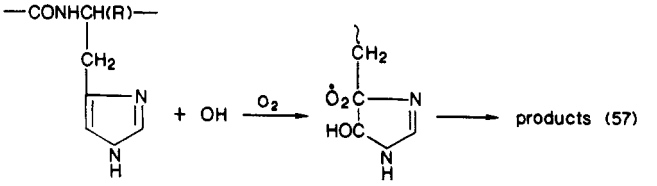


leads to formation of formylkinurenine as a major degradation product in oxygenated solution.[76] The



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% of the OH radicals add to the aromatic ring and about 60% react at the C_2-position[78] (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.,



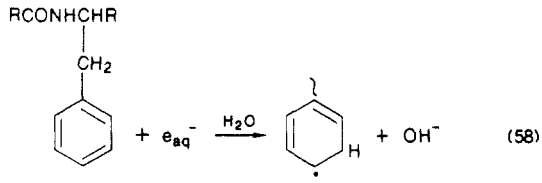
The yield for oxidative degradation of the histidine (imidazole) ring corresponds to G(-His) ~4 in dilute O_2-saturated solutions under γ-rays, and a complexity of degradation products is observed.[79] Among the major products of reaction (57) are asparagine and aspartic acid. The identification of imidazolylpyruvic 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 rela-

tively fast with k-values in the 5×10^9–10^{10} M^{-1} s^{-1} range.[60,69]

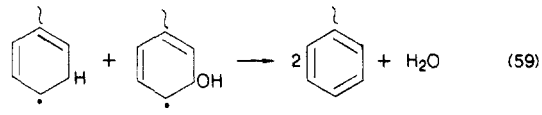
2. Oxygen-Free Solutions

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 reaction 58.[61,62,82]



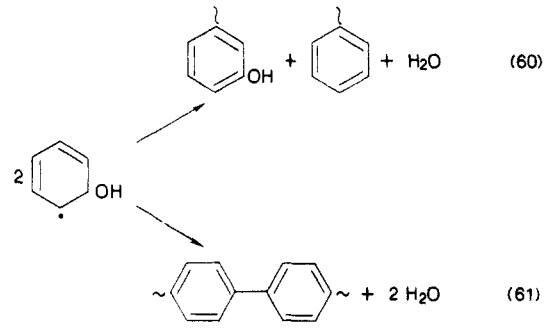
(58)

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 adds to the benzene ring via reaction 58.[82] Subsequent reactions of the e(H) adduct[83] formed in step 58 and the OH attack of step 51 above include the back reaction



(59)

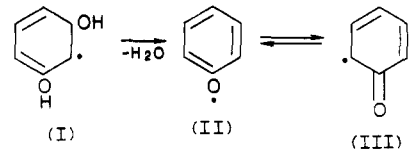
and



(60)
(61)

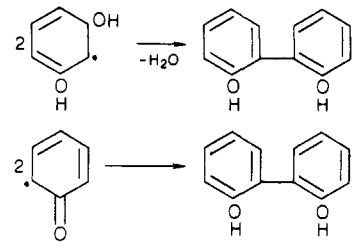
to give tyrosine, phenylalanine dimer, and their respective isomers.[84-86] Detailed gas chromatography–mass spectrometry (GCMS) studies of product yields in the γ-radiolysis of phenylalanine and its homopeptides in oxygen-free N_2O-saturated solution give G values of 0.50, 0.30, 0.5, and 1.5 for the formation of o-, m-, and 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 cross-linking between ^{14}C-labeled 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 reactions 60 and 61 also occurs in the radilysis of aqueous solutions of tyrosine and tyrosine peptides. A blue fluorescent product characteristic of dityrosine is observed in irradiated oxygen-free solutions of tyrosine, glycyltyrosine, and polytyrosine.[90,91] More recent studies[92] of the γ-ray induced cross-linking of tyrosine peptides in N_2O saturated solution have employed GCMS techniques for the isolation and identification of dimer products. The findings show that cross-linking 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]



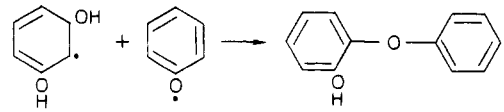
(62)

Dimerization of radicals I and/or III yield dityrosine



(63a)
(63b)

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



(64)

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 and III (eq 62) are present simultaneously, both C–C and C–O–C cross-links are formed, i.e.,

$$\text{T–OH} + \text{II} \rightarrow \text{HO–T–O–Phe}$$

$$\text{T–OH} + \text{III} \rightarrow \text{HO–T–Phe–OH}$$

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

$$\text{TrpH} + \text{TrpOH} \rightarrow 2\text{Trp} + \text{H}_2\text{O}$$

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}^- ($k \sim 5 \times 10^9$ M^{-1} s^{-1}) to give a radical product with two characteristic absorption bands, λ_{max} = 290 and 360 mm.[60,81] The nonprotonated ring is much less reactive towards e_{aq}^-; with a simple peptide such as glycylhistidine at 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

$$RSH + OH \rightarrow \dot{R}S + H_2O \quad (65)$$

where reaction 65 is essentially diffusion controlled with $k \simeq 10^{10}$ M^{-1} s^{-1}.[60,69] In acidic oxygenated solution the overall stoichiometry corresponds to[95-98]

$$2RSH + O_2 \rightarrow RSSR + H_2O_2 \quad (66)$$

In the γ-radiolysis of 10^{-3} M cysteine in O_2-saturated solution at pH 3, $G(-RSH) \simeq 10$, $G(RSSR) \simeq 5$, $G(H_2O_2) \simeq 5$. The mechanism in acidic solution involves a short chain:

$$\dot{R}S + O_2 \rightarrow R\dot{S}O_2 \quad (67)$$

$$R\dot{S}O_2 + RSH \rightarrow RSOOH + \dot{R}S \quad (68)$$

The hydroperoxide radical, HO_2, appears to be unreactive towards RSH. Observed products are formed via the subsequent chemistry

$$RSOOH + RSH \rightarrow RSSR + H_2O_2 \quad (69)$$

$$RSOOH + H_2O \rightarrow RSOH + H_2O_2 \quad (70)$$

$$RSOH + RSH \rightarrow RSSR + H_2O \quad (71)$$

A marked increase in the values of $G(-RSH)$, $G(RSSR)$, $G(H_2O_2)$ is observed with increasing pH above pH $\geq$ 5.[96,98] The evidence is that the thiolate ion ($RSH + OH^- \rightleftarrows RS^- + H_2O$) competes with oxygen (reaction 67) for thiyl radicals via

$$RS^- + \dot{R}S \rightleftarrows (\overline{RSSR}) \quad (72)$$

where the radical ion reacts in turn with oxygen to yield O_2^-

$$(\overline{RSSR}) + O_2 \rightarrow RSSR + O_2^- \quad (73)$$

It was proposed that O_2^- then generates additional $\dot{R}S$ via[98]

$$RSH + O_2^- \rightarrow \dot{R}S + HO_2^- \quad (74)$$

and that reaction 72–74 constitute the chain which gives $G(-RSH)$ values as high as 30 in the γ-radiolysis of 3×10^{-3} M cysteine solutions containing 2×10^{-4} M oxygen at pH 9. However, direct spectro-kinetic studies[99] have since shown that O_2^- (like HO_2) is essentially unreactive towards RSH with $k_{74} \sim 15$ M^{-1} s^{-1}. It would appear then that a propagation step other than reaction 74 is involved in the radiolytic oxidation of cysteine at high pH values.

Oxidation of the disulfide linkage of cystine by the hydroxyl radical is also a fast reaction ($k \simeq 1 \times 10^{10}$ M^{-1} s^{-1}) which involves ion-pair formation and dissociative OH addition[100,101]

$$RSSR + OH \rightarrow (RSSR)^+ + OH^- \quad (75)$$

$$RSSR + OH \rightarrow RSOH + \dot{R}S \quad (76)$$

where reactions 75 and 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 $G \simeq 1.7$ and $G \simeq 0.7$, respectively.[102]

Chemical and pulse radiolysis studies show that methionine in the peptide form reacts with OH primarily as a thioether.[100] Oxidative attack occurs predominantly at the sulfur locus to give an addition product ($k_{77} \sim 10^{10}$ M^{-1} s^{-1})

$$RSCH_3 + OH \rightarrow R\dot{S}(OH)CH_3 \quad (77)$$

which in oxygen-free solution breaks down in a complex series of reactions to yield a cation dimer and a sulfone.[100,103] 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 $G \simeq 3$.[105]

2. Oxygen-Free Solutions

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

$$RSH + e_{aq}^- \rightarrow \dot{R} + SH^- \quad (78)$$

is essentially quantitative with $k_{78} \simeq 10^{10}$ M^{-1} s^{-1} in neutral solution. The alanine radicals, $\dot{R}$, formed in reaction 78 are removed in turn via the abstraction reaction

$$\dot{R} + RSH \rightarrow R\dot{S} + RH \quad (79)$$

The $R\dot{S}$ radicals formed in reaction 79 and in the OH reaction 65 then dimerize ($2R\dot{S} \rightarrow RSSR$) to give $G(\text{cystine}) \simeq G(\text{alanine}) \simeq G(H_2S) \simeq 3$ in steady-state γ-radiolysis.[42,106] Pulse radiolysis studies[107] are in accord with the above formulation.

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

$$RSSR + e_{aq}^- \rightarrow R\overline{SS}R \quad (80)$$

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

$$R\overline{SS}R \rightarrow \dot{R}S + RS^- \quad (81)$$

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 2-methyl-2-nitrosopropane radicals also indicate a small contribution of[65]

$$RCH_2SCH_3 + e_{aq}^- \rightarrow \dot{R}CH_2 + CH_3S^- \quad (82)$$

$$RCH_2SCH_3 + e_{aq}^- \rightarrow RCH_2S^- + \dot{C}H_3 \quad (83)$$

IV. Main-Chain Chemistry of Proteins in Aqueous Solution

A. Oxygenated Solutions

The early studies of the oxidative degradation of the peptide chain in aqueous O_2-saturated solutions[45-48] 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

$$P\text{–}CONHCH(R)\text{–}P + OH \rightarrow P\text{–}CONH\dot{C}(R)\text{–}P + H_2O \quad (84)$$

$$P\text{–}CONH\dot{C}(R)\text{–}P + O_2 \rightarrow P\text{–}CONHC(\dot{O}_2)R\text{–}P \quad (85)$$

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 eq 13 (section II), i.e.,

$$\text{P–CONHCH(R)–P} + O_2 \rightsquigarrow \text{P–CONH}_2 + \text{RCO–P} + H_2O_2 \quad (86)$$

The attack of OH via reaction 84 was viewed as occuring 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% in oxygenated solution) was included in this early study. The keto acid products include glyoxylic, pyruvic, phenylpyruvic, α-ketoglutaric, oxalacetic plus traces of other unidentified α-keto acids to give a combined carbonyl yield of $G(>CO) \simeq 0.9$. The combined amide yield is appreciably higher with $G(\text{amide}) \simeq 1.25$. More detailed studies with model peptides[46–48,52] 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:

$$\text{P–CONHCH(R)–P} + O_2 \rightsquigarrow \text{P–CONH}_2 + \text{RCOOH} + \text{products} \quad (87)$$

The specific intermediate chemistry is given in section 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)

$$\text{PSSP} + \text{RSH} \rightarrow \text{PSH} + \text{PSSR} \quad (88)$$

$$\text{PSSR} + \text{RSH} \rightarrow \text{PSH} + \text{RSSR} \quad (89)$$

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, and 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 eq 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 $\sim\text{CONH}\dot{\text{C}}(\text{R})\sim$ 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 papain[112] and ribonuclease[113] suggest that ~20–30% 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 absorption,[116] 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 and 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.

B. 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

$$\text{P–CONH–P} + e_{aq}^- \rightarrow \text{P–}\dot{\text{C}}(\text{O}^-)\text{NH–P} \rightarrow \text{P–}\dot{\text{C}}(\text{OH})\text{NH–P} \quad (90)$$

occurs in competition with e_{aq}^- addition to the disulfide bond of cystine (section III.C) and the protonated histidine residue (section III.B). For example, pulse radiolysis studies of papain[112] and α-chymotrypsin[118] indicate that reaction 90 represents a major path for removal of 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 studies[122] 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, section II) represents a major chemical consequence of

$$\text{P–}\dot{\text{C}}(\text{OH})\text{NHCH(R)–P} \rightarrow \text{P–CONH}_2 + \dot{\text{C}}\text{H(R)–P} \quad (91)$$

the addition of e_{aq}^- to peptide carbonyls of histone H1 and protamine (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 $-\dot{C}(O^-)NH-$ 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 77 K 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 cm^2/Vs and $\sim$6 cm^2/Vs, respectively, whereas with "hydrated" polyglycine the corresponding values are $\sim$150 cm^2/Vs and $\sim$4 cm^2/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

In studies with papain[112,127] and glyceraldehyde-3-phosphate dehydrogenase, GAPDH,[128] three reaction modes for the radiation-induced oxidation of protein SH groups in oxygenated solutions have been formulated, i.e.,

$$PSH + OH \rightarrow P\dot{S} + H_2O \quad (92)$$

$$PSH + O_2^- \rightarrow P\dot{S} + HO_2^- \quad (93)$$

and the slower molecular process

$$PSH + H_2O_2 \rightarrow PSOH + H_2O \quad (94)$$

The radical reactions 92 and 93 are analogues of reactions 65 and 74 which were discussed in the section on cysteine and its simple model peptides (section III.C). Subsequent reaction of macromolecular $P\dot{S}$ with O_2 leads to formation of nonrepairable products which are presumed to be the sulfinic (RSOOH) and sulfonic (RSO_2H) derivatives.

$$P\dot{S} + O_2 \rightarrow PSO_2 \rightarrow \text{products} \quad (95)$$

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]

$$PSOH + RSH \rightarrow PSSR + H_2O \quad (96)$$

$$PSSR + RSH \rightarrow PSH + RSSR \quad (97)$$

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 $G(\text{inact})_{O_2} \simeq 4.8$ of which $G \simeq 3.5$ 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 $\sim$20% of the available OH radicals are involved in reaction 92 to yield nonrepairable 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 the reaction of H_2O_2 with PSH as formulated in reaction 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 $G(\text{inact})_{O_2} \simeq 0.12$. 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 than the SH groups of LDH which do not have this function but conserve three-dimensional structure through H bond formation.

The inactivation yield $G(\text{inact})_{O_2} \simeq 0.12$ obtained with LDH falls within the range of inactivation yields observed with most other enzymes $G(\text{inact})_{O_2} \simeq 0.1$ to $\simeq 0.5$ in oxygenated solution.[131,132] There are two main reasons for the generally low values of enzyme inactivation by ionizing radiation: (1) Only a few enzymes such as papain and GAPDH have active sites reactive toward H_2O_2. (2) The attack of OH radicals is relatively nonspecific 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

$$PSSP + e_{aq}^- \rightarrow P\overline{SS}P \quad (98)$$

absorption band at $\sim$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 $P\overline{SS}P$ adduct is short-lived and undergoes the dissociation

$$P\overline{SS}P \rightarrow P\dot{S} + PS^- \quad (99)$$

$$PS^- + H_2O \rightarrow PSH + OH^- \quad (100)$$

as is found with the simple disulfides, cystine, etc. The production of PSH by the dissociation reactions 99 and 100 has been observed with a number of aqueous proteins including papain[134] and Bowman–Birk proteinase inhibitor.[135] With papain, the dissociation reaction 99 contributes to the overall inactivation yield. The $P\overline{SS}P$ adducts of the second type are long lived and have the stability of $-\overline{S}-\overline{S}-$ ions observed in aqueous glasses.[125,136] Apparently the long-lived $P\overline{SS}P$ adducts have structures and environments which prevent dissociation. Enzymes that show a high yield of long-lived $P\overline{SS}P$ radicals with little or no short lived component are not significantly inactivated by e_{aq}^-.[133] It has been suggested that the long-lived $P\overline{SS}P$ species are ultimately removed through back reaction with radicals formed through OH attack at various side-chain and main-chain loci.[121]

The $P\dot{S}$ 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 noncovalent 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] and 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 cross-links and those that contain other (nonreducible) 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%) than cross-links derived from other types of radical–radical reactions. These other modes of radiation cross-linking 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)

$^{+}NH_3$-Lys-Glu-Thr-Ala-Ala-Ala-Lys-Phe-Glu-Arg
|
^{-}OOC-Ala-Ala-Ser-Thr-Ser-Ser-Asp-Met-His-Glu

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*-protein.

Studies of the radicals formed in the reactions of photogenerated OH radicals ($H_2O_2 \xrightarrow{h\nu} 2OH$) with *S*-peptide (using 2-methyl-2-nitrosopropane as the spin trap, eq 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/mol *S*-peptide (at 17 percent residual activity) correspond to Met ~0.65, Ala ~0.25, Ser ~0.25, Phe ~0.2, Lys ~0.2, His ~0.18, Glu ~0.15, Arg ~0.13, Thr ~0.1, Asp ~0.1. Column chromatography (on polyacrylamide gels) of the irradiated *S*-peptide (after mild hydrolysis at pH 3 for 30 min) 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 reexamination 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 section V.A 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 ($N_2O + e_{aq}^- \rightarrow N_2 + OH + OH^-$) the transient spectra from the reaction of OH with most proteins (for example, pepsin,[142] RNase,[113] lysozyme,[146] and α-chymotrypsin[118]) are relatively featureless and reflect the nonspecificity 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

$$X^- + OH \rightarrow X + OH^- \quad (101)$$

$$X + X^- \rightarrow X_2^- \ (X = CNS^-, Br^-, I^-) \quad (102)$$

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 study[143] the specificity of the reaction of $(CNS)_2^-$ radicals with tryptophan

$$TrpH + (CNS)_2^- \rightarrow TrpH^+ + 2\,CNS^- \quad (103)$$

$$TrpH^+ \rightarrow Trp^{\cdot} + H^+ \quad (104)$$

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 led to the tentative 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], and lactate dehydrogenase [cys].[130]

Transfer reactions between tryptophan radicals and tyrosine residues

$$Trp^{\cdot} + TyrOH \rightarrow TrpH + TyrO^{\cdot} \quad (105)$$

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

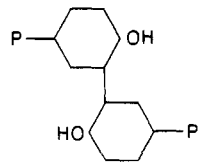
$$N_3^- + OH \rightarrow N_3^{\cdot} + H_2O \quad (106)$$

react selectively at tryptophan residues in neutral solution

$$TrpH + N_3^{\cdot} \rightarrow Trp^{\cdot} + H^+ + N_3^- \quad (107)$$

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 section III.B. There now is definite evidence that similar processes are involved in the radiation-induced dimerization of proteins. The dityrosyl cross-link



has been detected through use of its strong characteristic fluorescence spectrum with λ_{max} = 400 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 dityrosyl cross-links in these systems, G = 0.1, is quite low as compared to G(OH), their detection suggests that similar cross-linking 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 and conformations 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 collagen,[148] and thymus histone[12,13] show that damage to aliphatic residues accounts for 75, 85, and 80%, 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, and 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), and (c) a relatively low content of phenylalanine, tyrosine, and histidine which for total thymus histone account for less than 6 mol % of the amino acid content. Histone H1 has an aliphatic content of ~98 mol %. 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 4 M NaCl. The soluble histones were separated by centrifugation (70,000g) 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 mμ to confirm the absence of measurable amounts of DNA. Prior to irradiation the histone solutions (0.007%) were saturated with oxygen and were reoxygenated at 50 krad 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, and 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 ±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 and 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 86 and 87 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 krad range commonly used in biological studies. The analyses are based on the finding that reactive carbonyl groups are major products of protein oxidation initiated by OH attack as discussed in sections II.B and 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 (section III.B).

The γ-irradiation of deoxynucleoprotein in oxygen-free systems leads to the formation of DNA–protein cross-links 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, and H4 are the specific proteins involved in the radiation cross-linking reaction in chromatin.[18] Oxygen inhibits DNA histone cross-linking presumably by reacting preferentially with DNA and protein radicals.

VI. Solid-State Systems

A. Amino Acids

The identification of the reductive deamination reaction 2 in aqueous amino acid systems (section II.A) 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

$$^{+}NH_3CH(R)COO^{-} \rightsquigarrow {}^{+}NH_3\dot{C}(R)COO^{-} + H^{+} + e_s^{-} \quad (108)$$

$$e_s^{-} + {}^{+}NH_3CH(R)COO^{-} \rightarrow {}^{+}NH_3CH(R)\dot{C}\begin{matrix}O^{-}\\O^{-}\end{matrix} \rightarrow NH_3 + \dot{C}H(R)COO^{-} \quad (109)$$

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 $G(NH_3) \simeq G(RCOCOOH) + G(CH_2RCOOH) \simeq 5$.[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 $^{+}NH_3CH(R)\dot{C}OO^{2-}$ which dissociates on warming to yield $NH_3 + \dot{C}H(R)COO^{-}$.[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 109 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 studies[159–162] 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(amide) ≃ 3, G(propionic acid) ≃ 2, G(pyruvic acid) ≃ 1, and G(dimer) ≃ 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 study[165] in which irradiated solid *N*-acetylalanine was simply heated to 70–80 °C in the injection port of a gas chromatography 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]

$$3RCONHCHR_2 \rightsquigarrow RCONH_2 + CH_2R_2 + 2RCONH\dot{C}R_2 \quad (110)$$

$$RCONHCHR_2 \rightsquigarrow RCON{=}CR_2 + H_2 \quad (111)$$

where $RCONH\dot{C}R_2$ represents the long-lived radical products observed in the early ESR studies of γ-irradiated solid peptides.[166] Dimerization of these radicals 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.

$$RCON{=}CR_2 + H_2O \rightarrow RCONH_2 + R_2CO \quad (112)$$

ESR studies have since shown that the formation of amide and fatty acid via the stoichiometry of equation 110 involves the intermediate ionic processes:[162,167–169]

$$RCONHCHR_2 \rightsquigarrow (RCONHCHR_2)^{+} \rightarrow RCONH\dot{C}R_2 + H^{+} + e_s^{-} \quad (113)$$

$$e_s^{-} + RCONHCHR_2 \rightarrow R\dot{C}(O^{-})NHCHR_2 \xrightarrow{H^{+}} R\dot{C}(OH)NHCHR_2 \quad (114)$$

$$R\dot{C}(OH)NHCHR_2 \rightarrow RCONH_2 + \dot{C}HR_2 \quad (115)$$

$$\dot{C}HR_2 + RCONHCHR_2 \rightarrow CH_2R_2 + RCONH\dot{C}R_2 \quad (116)$$

to give the observed overall 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 $RCONH\dot{C}R_2$ as shown in eq 110 and 116. With tyrosine and cysteine peptides the long-lived radicals are at side-chain loci, viz ΦȮ and RṠ, 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.,

$$RCONHCH(R)COOH \rightsquigarrow [RCONHCH(R)COOH]^{+} \rightarrow RCONHCH(R)C\dot{O}O + H^{+} \quad (117)$$

The ionization step 117 is then followed by

$$RCONHCN(R)C\dot{O}_2 \rightarrow RCONH\dot{C}H(R) + CO_2 \quad (118)$$

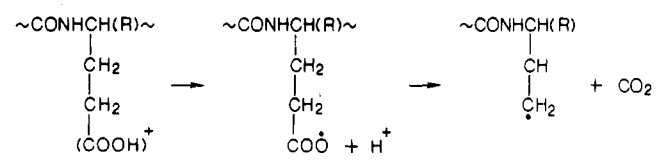
and the abstraction reaction

$$RCONH\dot{C}H(R) + RCONHCH(R)COOH \rightarrow RCONHCH_2(R) + RCONH\dot{C}(R)COOH \quad (119)$$

to yield the long-lived α-carbon radical[169] plus carbon dioxide and the *N*-acylamide as additional final products.[165,170] Both the decarboxylated radical, RCONHĊHR, and the α-carbon radical, RCONHĊ(R)COOH, have been spin-trapped on dissolution of γ-irradiated *N*-acetyl amino acids and dipeptides in aqueous solution of 2-methyl-2-nitrosopropane 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, ~CONHĊ(R)~, 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



(120)

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, and 83) the evidence from both product analysis[156] 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 and 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:

$$e_s^- + {}^+NH_3CH_2CO(NHCH_2CO)NHCH_2COO^- \rightarrow NH_3 + \dot{C}H_2(NHCH_2CO)NHCH_2COO^- \quad (121)$$

However, with triglycine in the basic form the deamidation reaction

$$e_s^- + NH_2CH_2CO(NHCH_2CO)NHCH_2COO^- N_a^+ \rightarrow NH_2CH_2CONH_2 + \dot{C}H_2CONHCH_2COO^- N_a^+ \quad (122)$$

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 state may also be involved in the radiolytic cleavage of the peptide chain,[174] i.e.,

$$RCONHCHR_2 \rightsquigarrow (RCONHCHR_2)^* \quad (123)$$

$$(RCONHCHR_2)^* + RCONHCHR_2 \rightarrow \dot{C}H_2R + RCONH_2 + RCONH\dot{C}R_2 \quad (124)$$

followed by the abstraction reaction 116 above. This reaction sequence gives the same product stoichiometry as the ionic mechanism given in eq 113–116.

C. Proteins

Studies of amino acid destruction in irradiated solid proteins[175–179] 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 aldehyde) with $G \sim 1$,[46,175,176,182] 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] lysozyme,[182] 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, given 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 $RCONH\dot{C}R_2$ 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 suggested[188] that electron capture via

$$RSSR + e_S \rightarrow R\dot{S}\dot{S}R \quad (125)$$

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 labeled hydrogen sulfide (HST) and hydrogen iodide (TI) which react essentially quantitatively over a period of a few hours to yield tritium labeled amino acids via[190]

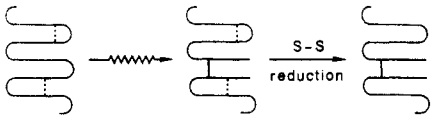
$$RCONH\dot{C}R_2 + HST \rightarrow RCONHCTR_2 + H\dot{S} \quad (126)$$

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 lyophollized, 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 explicity 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 reactions 113–116 has been obtained in studies of the effects of metal ions (Cu^{2+}, Fe^{2+}, and 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 $Cu^{2+} + e_s^- \rightarrow Cu^{1+}$ would be expected to eliminate any contribution of main-chain cleavage through reactions 114 and 115.

Although main-chain breaks are observed with fibrous proteins, studies of the effect of γ-rays on globular proteins (e.g., ribonuclease, lysozyme, and 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, cross-linking 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.,



(127)

where --- represent disulfide bondsof 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 cross-links shown in eq 127 would be formed through various combination reactions involving α-carbon radicals, $RCONH\dot{C}R_2$, and the side-chain radicals, e.g., $\Phi\dot{O}$ 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 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 corallary of this model for radiation damage summarized in eq 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 cross-link.

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