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REACTIONS OF THE PEPTIDE MAIN-CHAIN IN MODEL SYSTEMS

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August 1982

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Reaction Mechanisms in the Radiolysis of Peptides, Polypeptides and
Proteins I Reactions of the Peptide Main-Chain in Model Systems

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Abstract

The object of this review is to bring together and to correlate our present knowledge of products and mechanisms in the radiolysis of peptides, polypeptides and proteins in both aqueous and solid-state systems. Results obtained with various experimental techniques such as product analysis, competition kinetics, ESR spectroscopy and pulse radiolysis are included. Here in part I the emphasis is on the various radiation-induced reactions of the peptide main-chain in model systems. In Part II the emphasis is on the radiation chemistry of side-chain loci of the aliphatic, sulfur-containing, aromatic and other unsaturated amino acid residues in similar systems. And, in Part III this information on model systems is used in interpreting the mechanisms of chemical change in the radiolysis of proteins in aqueous solution and in the solid state.

[†] Consultant

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1. Introduction

Studies of the chemical actions of ionizing radiations on biochemical systems over the years have focussed primarily on the amino acid-peptide-protein system and the base-nucleotide-nucleic acid system. During the past decade, the major emphasis in radiation biology at the molecular level has been on the nucleic acid system because of its primary genetic importance. The radiation chemistry of DNA, RNA and their constituent compounds is now known in considerable detail.¹⁻³

In recent years it has become increasingly evident that the proteins which are associated with DNA in chromatin also have important functions in determining both the structural and transcriptional properties of the chromosome. The evidence is that the binding of chromosomal proteins to specific operator sites of DNA represses gene expression. The repressor-operator recognition process involves specific secondary bonds (hydrogen bonds, salt linkages, van der Waals couplings, etc.) between protein and nucleic acid. Chemical alteration of particular sites of either the protein or the nucleic acid component can lead to marked changes in the stability constant of the repressor-operator complex.⁴⁻⁷

Bacteriophage and other viruses provide useful model systems for obtaining information on the locus of radiation damage in nucleoprotein. Virus inactivation in aqueous solutions appears to arise almost exclusively from protein damaged caused by attack of radical

species derived from water.⁸⁻¹⁰ These findings are consistent with earlier studies which showed that when dilute aqueous solutions of thymus deoxynucleohistone are irradiated, relatively little destruction of the purine and pyrimidine bases is observed. The destruction of histone amino acids is the predominant reaction and this leads to a labilization of the DNA-histone binding.¹¹⁻¹³ The direct action of ionizing radiation on nucleoprotein appears to involve chemical change in both components; the production of DNA protein cross-links by both the direct and indirect action of radiation has been observed.⁸⁻¹⁴

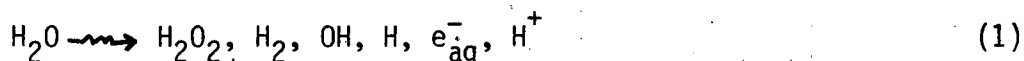
A detailed understanding of reaction products and reaction mechanisms in the radiolysis of proteins and related substances is becoming of increasing importance in radiation biology and related areas.¹⁵⁻¹⁷ The objective of this review is to bring together and to correlate our basic knowledge of products and mechanisms in the radiolysis of peptides, polypeptides and proteins in aqueous and solid-state systems. Results obtained with the various experimental approaches such as product analysis, competition kinetics, ESR spectroscopy and pulse radiolysis are included. Here in Part I, the emphasis is primarily on the various radiation-induced reactions of the peptide main-chain in model systems. In Part II the emphasis is on the chemistry of side-chain loci in similar systems. And, in Part III, this information on model systems is used in interpreting the mechanisms of product formation in the radiolysis of aqueous and solid proteins with particular reference to chromosomal proteins--the basic histones and the acidic non-histones.

2. Background Chemistry

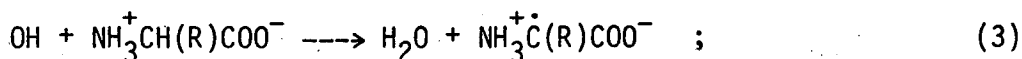
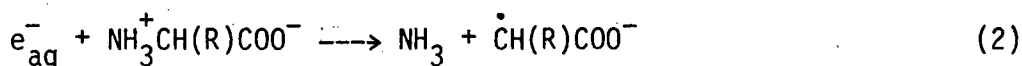
2.1 Aqueous Amino Acids

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.

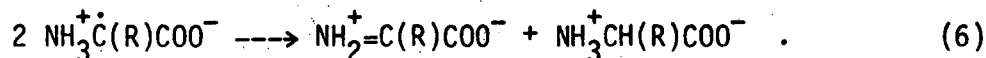
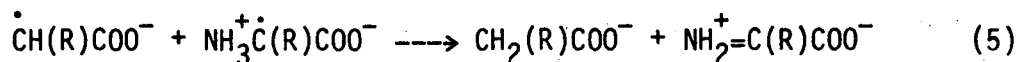
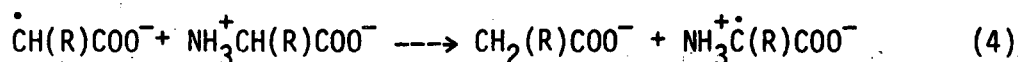
Chemical change in dilute aqueous solution is initiated by the radiation-induced decomposition of water^{18,19}



where e_{aq}^- represents the hydrated electron. For γ -rays and fast electrons the 100 eV yields (G) of the radical products correspond to $G(\text{OH}) \approx 2.8$, $G(\text{e}_{\text{aq}}^-) \approx 2.7$, $G(\text{H}) \approx 0.55$. The reactions of the major radical products e_{aq}^- and OH with the amino acids glycine and alanine in oxygen-free solution yields ammonia, keto acid and fatty acid as major products. Detailed chemical studies²⁰⁻²³ 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



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

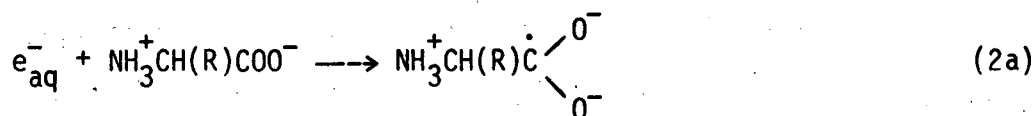


The overall stoichiometry of reactions 2-7 gives

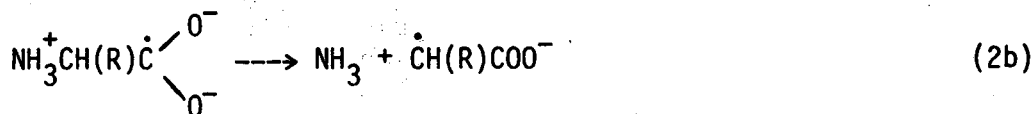
$$G(\text{NH}_3) \approx G(\text{RCOCOOH}) + G(\text{CH}_2\text{RCOOH}) \approx 5$$

which corresponds very closely to the experimentally observed values.

In an extension of these studies^{21,23} it was found that amino acids such as β -alanine and ϵ -aminocaproic do not undergo the reductive deamination reaction 2. Nor do the simpler aliphatic amines such as ethylamine. 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.

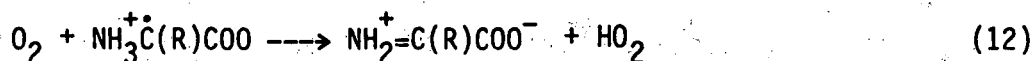
The radical products of reactions 2,3 have since been studied quite extensively by the pulse radiolysis technique.²⁴ Reactions 2a, 2b have also been observed in ESR studies of the reactions of photo-generated electrons with amino acids in aqueous glasses at low temperatures.²⁵

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}^- (ref. 26). 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.²⁸⁻³¹ With the amino acid cysteine the reactions of e_{aq}^- and OH occur exclusively at the SH function.³² Detailed reaction mechanisms involved in the radiolysis of the various amino acid side chains are treated in detail in Part II of this review.

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



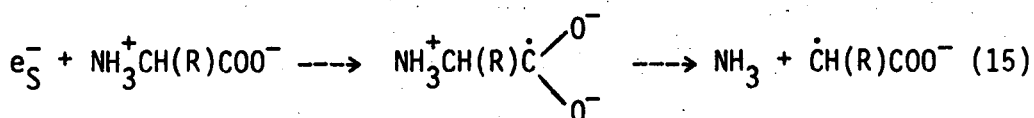
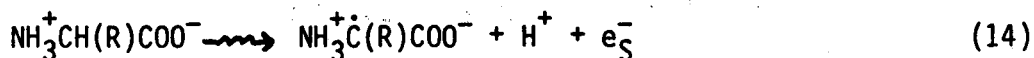
The OH reaction is not inhibited by O_2 and in the case of glycine and alanine the α -carbon radicals $\text{NH}_3^+\dot{\text{C}}(\text{R})\text{COO}^-$ formed in reaction 3 reacts with O_2 to yield ammonia and keto acid



The product stoichiometry in oxygenated solution is approximated by $G(\text{NH}_3) = G(\text{Carbonyl}) = G(\text{OH}) = 3.26$

2.2 Solid State Amino Acids

The identification of reaction 2 in aqueous systems 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.^{20,26} Ionic processes in these irradiated polar solids would then be represented by:



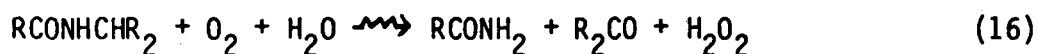
where reactions 14, 15 are the stoichiometric equivalents of reactions 2, 3 above. Reactions 4-6 which may occur in part in the solid are completed on dissolution of the irradiated solid in O_2 -free water.

It has since been established that keto acids and fatty acids are indeed formed as major products in the γ -radiolysis of solid glycine and alanine with $G(NH_3) \approx G(RCOCOOH) + G(CH_2RCOOH) \approx 5$ (ref. 33). Recent results obtained with ESR techniques provide physical evidence of reactions 7,8 in the solid state.^{34,35} On irradiation at 77°K the initially observed radical corresponds to the electron adduct $NH_3^+CH(R)\dot{C}OO^-$ which dissociates on warming to yield $NH_3 + \dot{C}H(R)COO^-$. The similarities between the radiation chemistry of these simpler amino acids in the solid state and in aqueous solution are quite striking.

Reductive deamination of most higher molecular-weight amino acids also yields free ammonia as a major product of radiolysis in the solid state. Ammonia yields from solid aspartic, serine, phenylalanine, cystine and cysteine, for example, are all in the range $G \sim 2$ to $G \sim 5$.^{26,33}

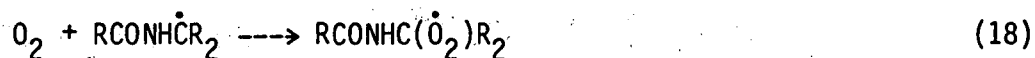
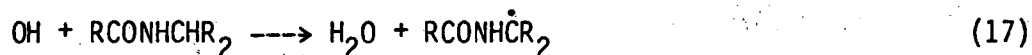
3. N-acyl amino acids

3.1 Oxygenated Solutions. Specific chemical evidence for oxidative degradation of the peptide main-chain through OH attack at α C-H bonds along the chain to yield amide and keto acid functions was first represented in terms of the overall stoichiometry³⁶

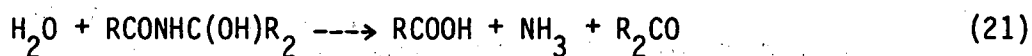
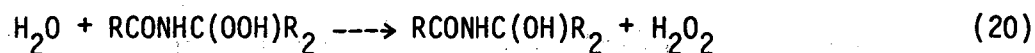
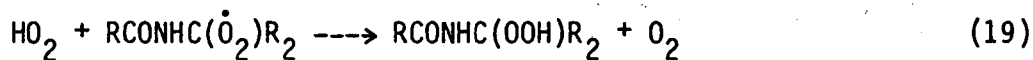


Much of the subsequent information on the detailed mechanisms of oxidative main-chain degradation has been derived from studies involving the N-acetyl derivatives of the simpler α -amino acids.

Radiolysis of N-acetyl glycine 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. The proposed reaction mechanism³⁷ includes the radiation-induced step 1 followed by:



The reducing species e_{aq}^- and H are scavenged preferentially by O_2 to give O_2^- and HO_2 which are related by the equilibrium $\text{HO}_2 \rightleftharpoons \text{H}^+ + \text{O}_2^-$. The subsequent steps were written:



If the radiation degradation of the peptide main-chain does occur predominantly through the sequence formulated in eq. 1, 17-21, then it is clear that ammonia and carbonyl yields with γ -rays should be in the

relationship $G(\text{NH}_3) \approx G(\text{R}_2\text{CO}) \approx G(\text{OH}) \approx 3$. Quantitative assays of the ammonia and carbonyl yields from a series of model peptide derivatives including acetylglycine, acetylalanine, glycine anhydride, etc., consistently show $G(\text{NH}_3) \approx 3$ for each system.³⁶⁻³⁸ 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 17-21. The carbonyl yields (keto acid plus aldehyde) are consistently low with $G(\text{R}_2\text{CO}) \approx 1$. 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 $\text{RCONHC}(\dot{\text{O}}_2)\text{R}_2$.

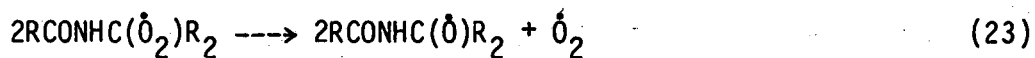
To obtain specific information on these questions, the radiolysis was carried out using ferric ion instead of O_2 as the scavenger of intermediate radicals formed by OH attack on N-acetylglycine and N-acetylalanine.³⁷ Heavy metal ions FeIII and CuII oxidize organic-free radicals in aqueous solution by electron transfer and by ligand transfer. Such reactions in the case of the peptide radical $\text{RCONHC}(\text{R}_2)$ would correspond to



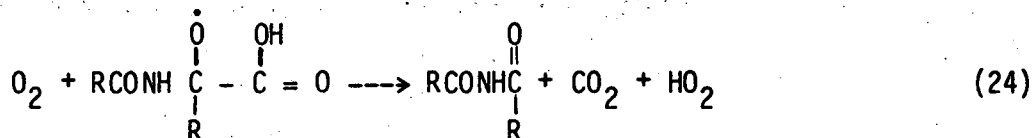
The oxidation product of reaction 22 is identical to the postulated product of reaction 20 and would then yield amide and carbonyl on hydrolysis. It was found that the γ -radiolysis of 0.1M N-acetylglycine and N-acetylalanine in O_2 -free solution containing .05 M FeIII gives

the product stoichiometry $-G(\text{peptide}) \approx G(\text{NH}_3) \approx G(\text{RCOCOOH}) \approx 3.2 \approx G(\text{OH}) + G(\text{H}_2\text{O}_2)$. In the presence of FeIII the values $G(\text{NH}_3)$ and $G(\text{RCOCOOH})$ are greater than $G(\text{OH})$ since molecular hydrogen peroxide formed in the radiation step, yields additional OH through reaction with FeII (generated in step 22) via $\text{FeII} + \text{H}_2\text{O}_2 \rightarrow \text{FeIII} + \text{OH} + \text{OH}^-$. The fact that much lower carbonyl yields are obtained with O_2 in place of FeIII shows then that the chemistry of removal of the peroxy radicals $\text{RCONHC}(\text{O}_2)\text{R}_2$ is indeed more complicated than that represented in reactions 18-21.

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.³⁸ 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 $\text{RCONHC}(\text{O}_2)\text{R}_2$ with HO_2 via step 19 occurs in competition with

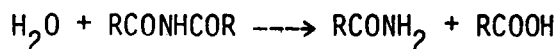


In the case of the N-acetyl amino acids, the alkoxy radicals formed in step 23 are removed in turn via



to yield a diacetamide derivative. In the case of N-acetylalanine the diacetamide product RCONHCOR is hydrolytically labile and under mild

differential hydrolysis in dilute base at room temperature is converted to acetamide and acetic acid.



The parent N-acetylalanine is stable under this condition. 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:³⁸

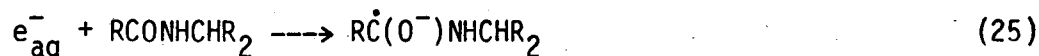
$$G(\text{CH}_3\text{COCO} + \text{CH}_3\text{CHO}) \sim 1, G(\text{CH}_3\text{COOH}) \approx 2.5, G(\text{CO}_2) \approx 2$$

The formation of several compounds with different oxidation states as initial products is frequently observed in oxidation processes involving peroxy radicals.³⁹

Evidence for reactions akin to step 24 have been identified in the radiolytic oxidation of the polypeptide main-chain (Sec. 4.2).

3.2 Evacuated Solutions

The chemical evidence is that in the absence of O_2 and other electron scavengers, the hydrated electron, e_{aq}^- , formed in the radiation induced step 1 adds to the carbonyl group of the peptide bond⁴⁰

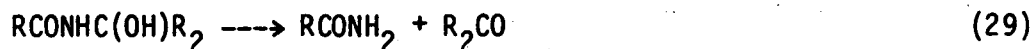
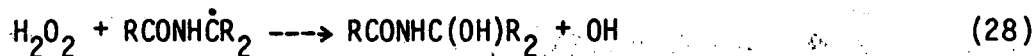


More recent studies of reactions 25, 26 using pulse radiolysis techniques have established $pK_a \geq 12$ for the equilibrium reaction 26.⁴¹

A major path for removal of $\dot{R}C(OH)CHR_2$ radicals appears to be the reconstitution reaction^{26,40}



where $RCONH\dot{C}R_2$ represents the product of OH attack at the α C-H position via step 17 above. Product analysis reveals little net radiation chemical degradation of the peptide main-chain in dilute O_2 -free solution. For example, the γ -radiolysis of neutral, O_2 -free solutions of N-acetylglycine and N-acetylalanine at solute concentrations below ~ 0.1 M gives $G(\text{amide}) \approx G(\text{carbonyl}) \approx 0.5$. The formation of amide and carbonyl (keto acid plus aldehyde) in low yield in these systems is attributed to the further oxidation of a small fraction of the $RCONH\dot{C}R_2$ radicals through, for example



where the H_2O_2 in reaction 28 is produced with $G = 0.8$ in the primary step 1.

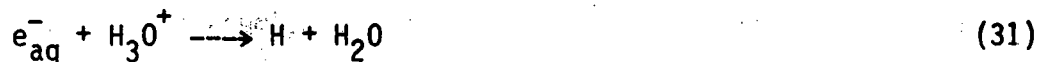
Addition of an electron scavenger such as N_2O at concentrations sufficient to remove e_{aq}^- preferentially via



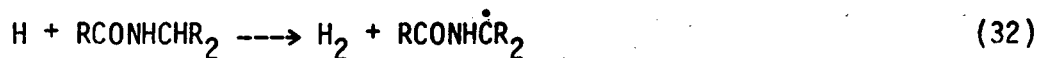
eliminates the possibility of reconstitution via reaction 27 and in the case of N-acetylglycine results in the production of the diamino-succinic acid derivatives (DA) through the dimerization (cross-linking) reaction



with $G(\text{DA}) \cong 2.5 \cong [G(\text{OH}) + G(e_{\text{aq}}^-)]/2$. Similarly in acid solutions where e_{aq}^- is converted to H

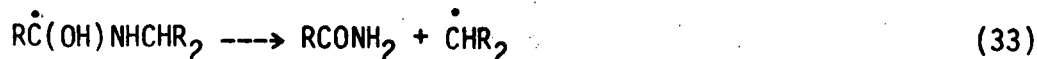


which also abstracts at the α C-H position



the diaminosuccinic yield is again approximated by $G(\text{DA}) = [G(\text{OH}) + G(e_{\text{aq}}^-)]/2 = 2.5$.⁴²

Neither the chemical-product analysis nor the pulse radiolysis data referred to above give evidence for any appreciable dissociation of the carbonyl-electron adduct formed in reaction 26, i.e.,



in peptide solutions at concentration below 0.05-0.1 M. However, if the rate of dissociation of $\dot{R}C(OH)NHCHR_2$ in the first-order reaction is relatively slow, then such dissociation would be favored at low dose rates since the competing reconstitution reaction 27 is of second order.

Evidence for main scission of simple peptides via reaction 33 has recently been obtained in studies in which t-nitrosobutane is used as a radical scavenger⁴³ e.g.:

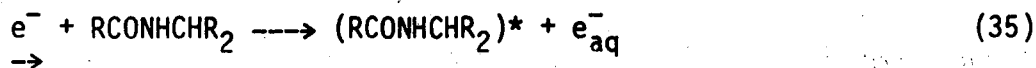


The more stable radical products of reaction 34 are then identified by ESR spectrometry.

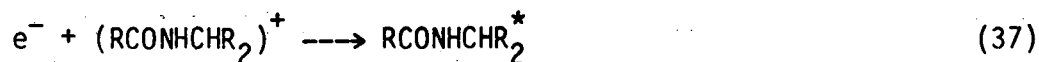
Chemical studies of N-acylamino acid solutions at concentrations above ~0.1 M indicate that chemistry in addition to that initiated by reactions of e_{aq}^- and OH becomes involved at the higher solute concentrations.⁴⁴ For example, in the γ -radiolysis of O_2 -free solutions of N-acetylalanine it is found that the yield of (amide) ammonia increases rapidly with increasing peptide concentration from $G(NH_3) \approx 0.4$ at 0.1M to $G(NH_3) \approx 2.8$ at 2.5M and then tends to level off at higher concentrations. This increase in $G(NH_3)$ is not accompanied by a corresponding increase in the yield of carbonyl products which is essentially constant at $G(>CO) \approx 0.5$ over concentration range 0.1 to 3M. Propionic acid is the principle concomitant product associated with this increase in the amide yield; the fatty

acid yield increases from $G < 0.1$ to $G \sim 2$ over the same concentration range. Addition of various second solutes of concentrations sufficient to preferentially scavenge e_{aq}^- and OH has relatively little effect on this elevated amide yield in concentrated aqueous solutions. All of the data en toto suggest that a new reaction mode(s) sets in as the peptide concentration is increased above $\sim .05M$.

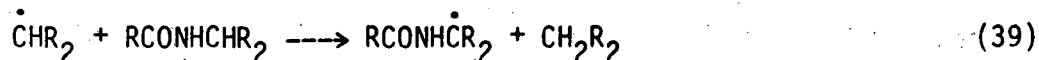
There is evidence⁴⁴⁻⁴⁶ that the production of amide and fatty acid in concentrated solution may involve excited molecular states $(RCONHCHR_2)^*$ formed through solute interaction with "sub-excitation" electrons⁴⁷



and/or through charge transfer reactions⁴⁸

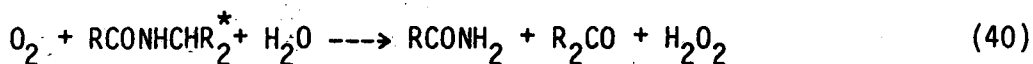


The ensuing chemistry is represented by



to give $G(\text{amide}) = G(\text{fatty acid}) = 2$.

Aromatic compounds are known to be effective scavengers of excited molecular states provided an energy level of the aromatic is lower than that of the excited species. It has been found that naphthalene sulfonic acid, benzoic acid and benzaldehyde are effective at millimolar concentrations in quenching the formation of amide ammonia in 2M acetylalanine. Phenol and benzene sulfonic acid on the otherhand are without effect even at high concentrations. Naphthalene sulfonic acid benzoic acid and benzaldehyde have lower triplet levels as compared to phenol and benzene sulfonic acid. The change from quenching to non-quenching occurs between benzoic acid ($27,200 \text{ cm}^{-1}$) and phenol ($28,500 \text{ cm}^{-1}$). In other words the energy of the excited state RCONHCHR_2^* corresponds to $\sim 28,000 \text{ cm}^{-1} = 80 \text{ kcal} = 3.5 \text{ eV}$.⁴⁶ Chemical quenching of RCONHCHR_2^* by O_2 and FeIII in concentrated N-acetylalanine solutions via the stoichiometries

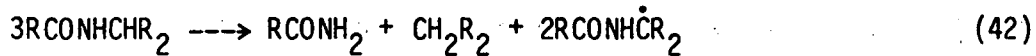


has been invoked⁴⁵ to account for the production of amide ammonia with $G(\text{NH}_3) \sim 5$ in 2M acetylalanine solution containing O_2 or FeIII (cf. sec. 3.1).

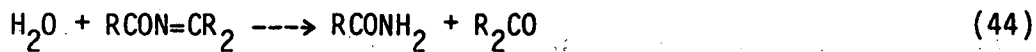
3.3 Solid State

Main-chain cleavage with formation of amide and fatty acid as major products was first identified in radiation chemical studies of the N-acylamino acids.⁴⁹ For N-acetylglycine and N-acetylalanine

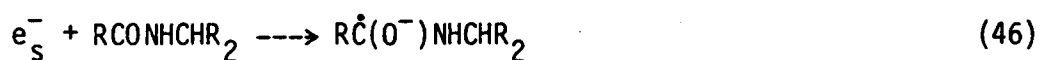
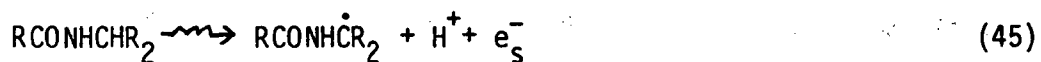
products yields measured after hydrolysis correspond to $G(\text{amide}) \approx 3$, $G(\text{fatty acid}) \approx 2$, $G(\text{keto acid}) \approx 1$, $G(\text{diamino acid}) \approx 1$. The products are accounted for in terms of the stoichiometries:

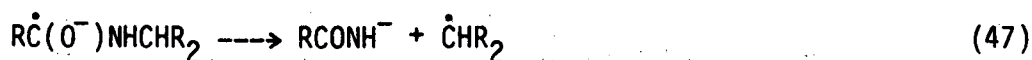


where the radical products of equation 42 represent the long-lived free radicals observed by ESR spectroscopy. The dehydropeptide formed in 43 reacts with water on hydrolysis to form amide and keto acid



The yield for total amide production has been determined for a series of aliphatic, aromatic and sulfur-containing amino acids in the N-acetyl form.⁵⁰ In the case of the aliphatic series, the length of the side chain has relatively little effect on the yield of main-chain degradation. The effect of aromatic groups of acetyl phenylalanine and tyrosine is to quench in part the production of amide function. The presence of the sulfur moiety of methionine appears to have little effect on the cleavage reactions. ESR studies of γ -irradiated N-acetylamino acids at low temperature suggest that the stoichiometry of equation (42) arises from the intermediate processes^{51,52}





The peptide electron-adduct, $\text{RC}(\text{O}^-)\text{NHCHR}_2$ is observed initially at 77°K. On warming, the dissociation step 47 and then the abstraction step 48 can be followed. Similar findings have been obtained with photo-generated electrons in aqueous glasses at low temperature.⁵²

On the other hand, chemical studies of the role of ionic processes in the γ -radiolysis of solid peptides suggest that such processes may not be of major importance at room temperature. In these studies,⁵⁰ advantage was taken of the fact that the simpler N-acetylamino acids can be prepared in the form of a clear glassy solid at room temperature. In the case of N-acetylalanine the glass has the composition $\text{RCNHCH(R)COONa} \cdot 2\text{H}_2\text{O}$ and gives product yields that are essentially the same as those obtained in the γ -radiolysis of solid polycrystalline acetylalanine, i.e., $G(\text{NH}_3) \approx 3$, $G(\text{propionic}) \approx 1.8$. Chloracetate ion which has been shown to be an effective electron scavenger ($e^- + \text{RCI} \longrightarrow \text{R} + \text{Cl}^-$) in other polar glasses is soluble as the sodium salt in the N-acetylalanine glass. On irradiation of this system it is found that $G(\text{Cl}^-)$ increases rapidly with chloracetate concentration from 1 to 12 mole percent which represents the solubility limit. A reciprocal yield plot of the data gives a limiting value of $G(\text{Cl}^-) \approx 3$ which is a reasonable value for ion-pair production in this system. The amide ammonia yield, however, remains essentially constant at $G(\text{NH}_3) \approx 2.9$ over the entire chloracetate concentration range. The results are not in support of reductive deamidation by e^- via steps 46, 47 in this particular systems at room temperature.

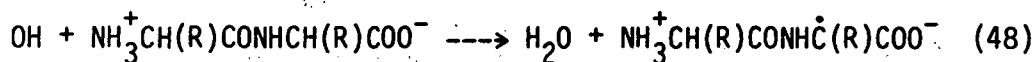
Also, if polycrystalline N-acetylalanine is irradiated at 77°K and subsequently warmed to room temperature prior to chemical analysis then the observed⁵⁰ propionic acid yield drops from $G(\text{RCOOH})_{290^\circ\text{K}}$ to $G(\text{RCOOH})_{77^\circ\text{K}} \leq 0.1$. The amide ammonia yield shows a corresponding drop from $G(\text{NH}_3)_{290^\circ\text{K}} = 3.2$ to $(\text{NH}_3)_{77^\circ\text{K}} = 1.3$. The yields of carbonyl products are unchanged with $G(>\text{CO})_{273^\circ\text{K}} = 1.3 = G(>\text{CO})_{77^\circ\text{K}}$ and in fact account for essentially all the amide ammonia produced at 77°K. The chemical consequences of reactions 46, 47 appear to be relatively unimportant under the conditions in which they were observed by ESR spectroscopy.

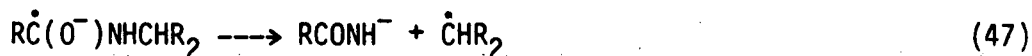
Reactions of excited molecular species RCONHCHR_2^* as discussed in section 3.3 also gives the product stoichiometry of reactions 42, 43 and at the present time cannot be ruled out as major reaction intermediates in the radiolysis of solid peptides at room temperature.

4. Oligopeptides and polypeptides

4.1 Oxygenated Solutions

Rate constants for reaction of OH with N-acetyl glycine and N-acetylalanine are $\sim 10^2$ times the rate constants for reaction of OH with the corresponding free amino acid zwitterion. Hence, it was anticipated that the reactions of OH with the dipeptide derivatives of glycine and alanine would occur preferentially at the peptide C-H linkage:





The peptide electron-adduct, $\dot{\text{R}}\text{C}(\text{O}^-)\text{NHCHR}_2$ is observed initially at 77°K. On warming, the dissociation step 47 and then the abstraction step 48 can be followed. Similar findings have been obtained with photo-generated electrons in aqueous glasses at low temperature.⁵²

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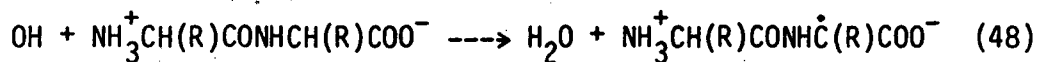
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4. Oligopeptides and polypeptides

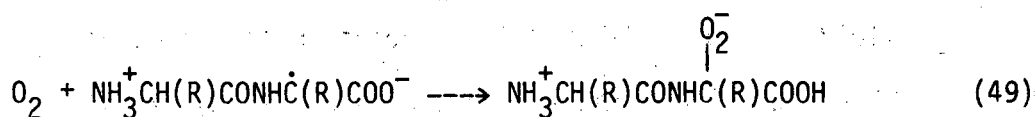
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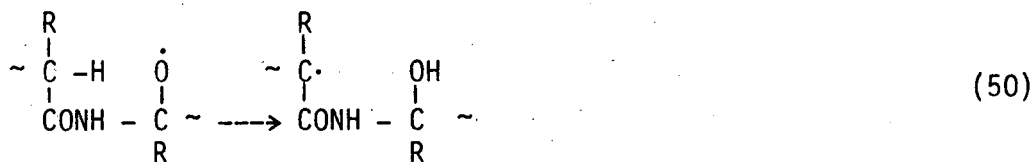


The chemical evidence⁵³ is that ~90 percent of the OH radicals are so removed. With the tri, tetra, etc. derivatives the reaction of OH at peptide C-H linkages is essentially quantitative. The formation of "peptide" radicals $\sim\text{CONH}\dot{\text{C}}(\text{R})\text{CONH}\sim$ through the reaction of OH with oligopeptides has been extensively studied by pulse radiolysis techniques.⁵⁴

In the presence of oxygen



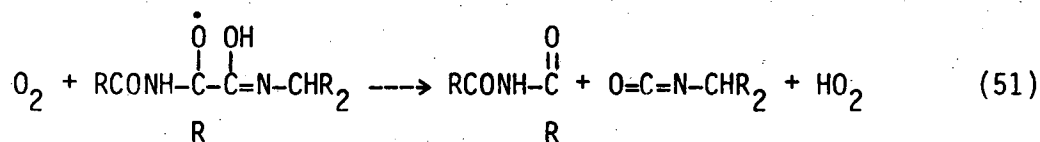
The subsequent chemistry is in part quite analogous to that observed in the radiolytic oxidation of N-acetylamino acids via the sequence 17-24 to yield amide and the lower fatty acid. However, with the oligopeptides the amide yield approaches $G(\text{NH}_3) \approx 5$ which is considerably higher than the value $G(\text{NH}_3) \approx 3 = G(\text{OH})$ observed with the N-acetylamino acids. The chemical evidence is that with oligo and polypeptides an intramolecular reaction occurs.



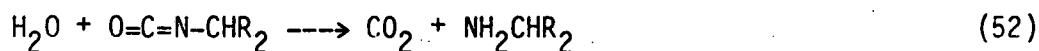
in competition with reaction 24. This leads to formation of additional ammonia and keto acid. With diglycine the major product stoichiometries correspond to $G(\text{NH}_3) = 4.8$, $G(\text{HCOOH}) = 1.7$, $G(\text{CHOCOOH}) = 1.9$.⁵³

With the N-acetylamino acids reaction of type 50 can only occur intermolecularly and is of negligible importance in competition with reaction 24. In agreement with reaction 50 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 $G(>CO) \approx 2$ with $G(NH_3) \approx 4.8$.⁵³

Product yields in the γ -radiolysis of polyalanine are consistent with the reaction sequence formulated above, i.e., $G(NH_3) \approx 4.0$, $G(RCOCO_2H) \approx 1.2$, $G(RCOOH) \approx 3.0$, $G(CO_2) \approx 2.4$.³⁸ With polyalanine at molecular weight ≈ 3000 it must be assumed that alkoxy-radical formation via reaction 17, 18, 23 must occur more or less at random along the peptide chain. The equivalent of reaction 24 then must involve an adjacent peptide bond (enol form), i.e.,



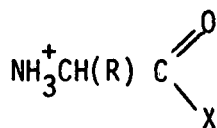
where



follows essentially instantaneously.

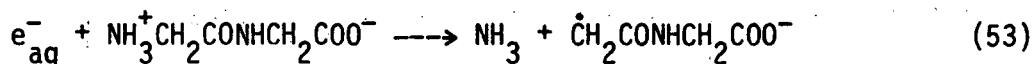
4.2 Evacuated Solutions

In extending the earlier chemical studies of the reductive determination of the α -amino acids by e_{aq}^- via step 2, 2a,b (sec. 2.1) it was also found that such deamination is a general and characteristic reaction of compounds containing the grouping

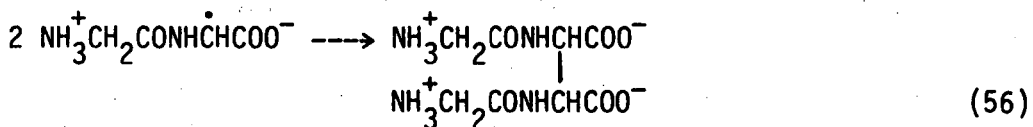
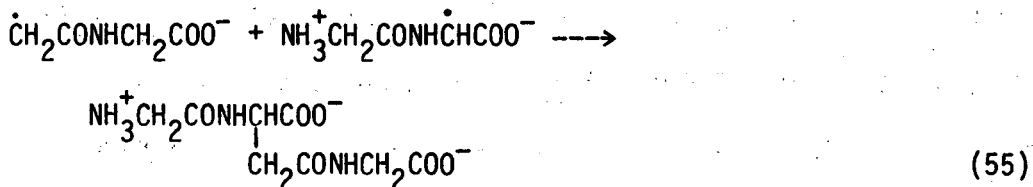
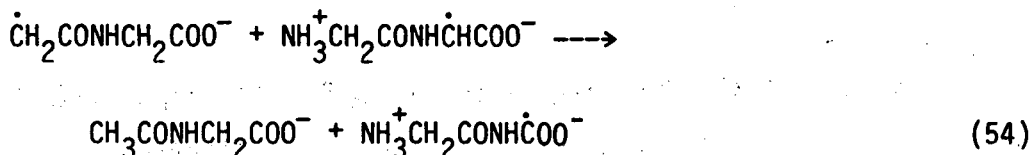


where X represents O^- , OH, OR, NHR, etc. ^{22,23,55,56}

With di and triglycine for example $G(\text{NH}_3) \approx 3 \approx G(\text{e}_{\text{aq}}^-)$, i.e.,



is essentially quantitative for both di and triglycine. Reaction of OH occurs preferentially along the chain away from the N-terminal residue as formulated in step 48. Subsequent steps in O_2 -free solution include

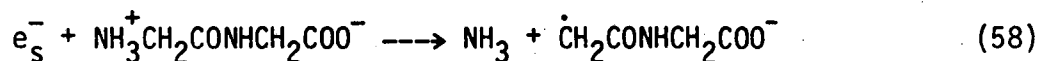


The combined yield of products derived from the $\dot{\text{C}}\text{H}_2\text{CONHCH}_2\text{COO}^-$ radical, i.e., acetylglycine (reaction 54) and aspartic acid derivative (reaction 55) are markedly dependent on the glycyglycine concentration. For example, in 0.5M solution the acetylglycine and aspartic

derivative are produced in essentially the same yield. However, in 1M solution $G(\text{acetylglycine}) \approx 2.9$, $G(\text{aspartic}) \approx 0.3$. The cross-linking reaction represents the major path for radical removal in 1.0M solution with $G(\text{diaminosuccinic}) \approx 1.7$.^{55,56} The free ammonia yield from dilute tetraglycine solutions is somewhat less than $G(e_{\text{aq}}^-)$, i.e., $G(\text{NH}_3)_f \approx 2.4$ and with polyalanine the free ammonia yield decreases to $G(\text{NH}_3)_f \leq 0.3$. There is, however, no corresponding increase in the amide ammonia with increasing chain length. This indicates that electron addition along the chain does not lead to reductive deamidation (cf. sec. 3.2). Similar conclusions have been arrived at from pulse radiolysis studies^{57,58} which also provide evidence for intra-chain electron transfer that is concentration dependent.

4.3 Solid Systems

Free ammonia and the corresponding acetyl derivatives are major products in the γ -radiolysis of solid di, tri, and tetrapeptide derivatives of glycine and alanine.^{55,59} Product yields range from $G(\text{NH}_3)_f \approx 4.5$, $G(\text{acetylglycine}) \approx 3.2$ with solid glycine to $G(\text{NH}_3)_f = 2.5$, $G(\text{acetyltriglycine}) = 2$ with solid tetraglycine. The observed chemistry is consistent with the reaction



followed by reactions 55-56 on dissolutions of the irradiated solid in O_2 -free water.

The decrease in $G(NH_3)_f$ from 4.5 with diglycine to ~ 2.3 with tetraglycine is attributed to an increasing probability for capture of e_s^- at peptide $C=O$ bonds along the chain. With polyglycine and polyalanine $G(NH_3)_f \leq 0.4$. At the same time, the production of amide ammonia gradually increases from $G(\text{amide}) \approx 0.4$ with the dipeptide to $G(\text{amide}) \approx 1.8$ with the polypeptide.⁵⁹ These results are consistent with a competing reductive deamidation by e^- as formulated in reactions 46,47.^{59,60} Here again the alternative is the excited molecule reaction sequence 35-39. Further work should clear up these uncertainties in the intermediate processes.

A detailed chemical study^{49,50} of products formed in the γ -radiolysis of solid polyalanine gives as major products $G(\text{amide}) \approx 3.1$, $G(\text{propionic acid}) \approx 1.8$, $G(\text{carbonyls}) \approx 1.4$. These findings are wholly consistent with the overall reaction stoichiometries observed with solid N-acetylalanine as summarized in sec. 3.3.

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