LBL-8928 c. 2
UC-4

THE RADIATION CHEMISTRY OF AMINO ACIDS, PEPTIDES AND PROTEINS IN
RELATION TO THE RADIATION STERILIZATION OF HIGH-PROTEIN FOODS

Warren M. Garrison

RECEIVED
LAWRENCE
BERKELEY LABORATORY

JUN 14 1979

March 1979

LIBRARY AND
DOCUMENTS SECTION

Prepared for the U. S. Department of Energy
under Contract W-7405-ENG-48

TWO-WEEK LOAN COPY

*This is a Library Circulating Copy
which may be borrowed for two weeks.
For a personal retention copy, call
Tech. Info. División, Ext. 6782*

LBL-8928 c. 2

Warren M. Garrison

ABSTRACT

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. The present evaluation of the radiation chemistry of amino acids, peptides and proteins outlines the various radiation-induced processes which lead to amino acid degradation and to the synthesis of amino acid derivatives of higher molecular weight. Among the latter are the α, α' -diamino dicarboxylic acids which are formed as major products in the radiolysis of peptides both in aqueous solution and in the solid state. The α, α' -diamino acids are of particular interest as irradiation products because they represent a class of compounds not normally encountered in plant and animal protein sources. Such compounds have, however, been isolated from certain types of bacteria and pathogenic toxins. All of the available data strongly suggest that the α, α' -diamino acids are produced in significant yield in the radiation sterilization of high protein foods. The importance of initiating extensive chemical and biological studies of these and of other high molecular weight products in irradiated food is emphasized.

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

THE RADIATION CHEMISTRY OF AMINO ACIDS, PEPTIDES AND PROTEINS IN
RELATION TO THE RADIATION STERILIZATION OF HIGH-PROTEIN FOODS

Warren M. Garrison[†]
Materials and Molecular Research Division
Lawrence Berkeley Laboratory, University of California
Berkeley, California 94720

1. Introduction

The use of ionizing radiation for the preservation of foods offers extraordinary possibilities for greatly increasing the availability of foodstuffs throughout the world. Broad economic and social advantages would be derived from the development of a successful food irradiation technology.¹⁻³

In recent years it has been shown that the radiation sterilization of meats in the frozen state in the absence of oxygen yields products with essentially the same taste, aroma and color as the unirradiated samples.^{4,5} The question of the wholesomeness of irradiated high-protein foods is receiving careful consideration. Extensive biological testing of the nutritional and toxicological aspects of the wholesomeness problem are in progress in a number of countries.¹⁻⁴

Chemical identification of products formed in the radiolysis of food constituents offers an important potential source of information on the question of whether or not toxic or other deleterious compounds are formed. Major chemical components of meat include water, protein and lipid in the approximate percentages of 65, 25 and 15 percent respectively.

[†]Present address: P. O. Box 744, Alamo CA 94507

Radiation chemical change in the protein and lipid fractions from energy absorbed directly in the organic component and from the indirect action of reactive radical species formed in the radiation decomposition of water. We review here the radiation chemistry of amino acids, peptides and protein in aqueous solution and in the solid state with particular reference to the subject of product identification.

Although the concentration of free amino acids in biological tissue is relatively low, we include a discussion of their radiation chemistry because such studies have provided basic information in the development of our understanding of the more complex radiation chemistry of peptides and protein.

2. Amino Acids in Aqueous Solution

The radiolysis of water is well described.^{6,7} The formation of major decomposition products can be summarized in terms of the formulation.

where e_{aq}^- represents the hydrated electron. For γ -rays and fast electrons the 100 eV yields, (G), of the free 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, of e_{aq}^- and OH, with the simpler α -amino acids, glycine and alanine in oxygen-free solution yields ammonia, keto acid and fatty acid as major products.⁸⁻¹² Detailed chemical studies of these systems, including the use of added second solutes for the preferential scavenging of e_{aq}^- and OH, led to formulation of the reaction scheme¹²⁻¹⁶

followed by

The labile imino acid derivative produced in the disproportionation steps 5,6 hydrolyzes spontaneously

The overall stoichiometry of reactions 2-7 gives

The yield of higher molecular-weight products from glycine and alanine is low. Radicals of the type $\text{NH}_3^+\dot{\text{C}}(\text{R})\text{COO}^-$ disproportionate almost quantitatively as shown in steps 5,6. In the case of glycine a small fraction of the $\text{NH}_3^+\dot{\text{C}}\text{HCOO}^-$ radicals undergoes dimerization to yield α,α' -diaminosuccinic acid¹²

Since neither ethylamine nor β -alanine were found to undergo the reductive deamination reaction 2, it was proposed¹⁴⁻¹⁶ that e_{aq}^- adds to the C=O bond of the α -amino acid and that the reduced intermediate then dissociates.

The radical products of reactions 2,3 have since been studied quite extensively by the pulse radiolysis technique.¹⁷ The reaction sequence 2a has also been observed in ESR studies of the reactions of photo-generated electrons with amino acid in aqueous glasses at low temperatures.¹⁸

With the aliphatic amino acids of higher molecular weight, i.e., with α -aminobutyric acid, valine, leucine etc. The reductive deamination reaction 2(2a) continues to represent a major path for removal of e_{aq}^- .¹⁹⁻²² However, with the longer aliphatic side-chains, the analogues of reactions 3,4 are no longer confined to the C-H bond at the α -carbon position. Other sites along the chain become involved. The radicals so formed react as typical aliphatic carbon radicals and preferentially dimerize rather than disproportionate via reactions 5b. With α -aminobutyric acid,⁵ for example, both α, α' -diamino suberic acid

and α, α' -diaminomethyl pimelic acid

are formed as major products with a combined yield of $G \approx 3$.¹⁹

Studies of product yields in the radiolysis of phenylalanine in neutral oxygen-free solution show that a major fraction of e_{aq}^- is removed via the reductive deamination reaction 2.^{23,24} Recent pulse radiolysis studies indicate that ~50 percent of e_{aq}^- reacts via 2 while the remainder adds to the aromatic ring.²⁵ The OH radical reacts both at the α -carbon position via 3 and also through ring addition.^{23,26,27} Comparison of the

observed yields for phenylalanine destruction, $G(-Ph)$ with the yields of phenylpropionic acid, phenylpyruvic and tyrosine indicate that unidentified higher molecular-weight products are produced in appreciable yield to account for $G(-Ph) \approx 5$.²³ By analogy with results obtained in radiolysis studies of aqueous benzene,^{28,29} one of these (dimer) products would correspond to the α, α' -diamino acid.

Both e_{aq}^- and OH react with tryptophan, and histidine almost quantitatively through ring addition.³⁰⁻³³ However, chemical studies show that the net destruction of solute is considerably less than $G(OH) + G(e_{aq}^-) \approx 5$. For example with tryptophan $G(-M) < 1$ in oxygen-free solution.³¹ The evidence is that in the radiolysis of unsaturated ring systems a reconstitution reaction is involved^{31,34,35}

The presence of a second solute at concentrations sufficient to preferentially scavenge OH in these systems leads to an enhancement in the yield for solute destruction since the possibility for self protection through water elimination (reaction 10) is precluded i.e.³⁵

The radiation chemistry of cysteine ($\text{NH}_3^+\text{CH}(\text{CH}_2\text{SH})\text{COO}^-$) and other aliphatic thiols in dilute oxygen free solution occurs exclusively at the SH group^{36,37}

followed by

to give $G(\text{cystine}) \simeq G(\text{alanine}) + G(\text{H}_2\text{S}) \simeq 3$. Pulse radiolysis studies are in accord with the above formulation.^{38,39} Similar chemistry has been observed with penicillamine. The dimer product cystine (RSSR) in equation (17) is of course, an α, α' -diamino acid.

It is, in fact, the only α, α' -diamino acid found naturally in food proteins.

3. Amino Acids in the Solid State

The production of major products in the γ -radiolysis of the simpler α -amino acids in the solid state has been shown to be consistent with the reaction sequence:^{13,21,22}

followed by:

and

on dissolution of the irradiated solid in O_2 -free water. With glycine and alanine at room temperature: $G(\text{NH}_3) \simeq 5$, $G(\text{RCOCCOOH}) \simeq 2.5$, $G(\text{RCH}_2\text{COOH}) \simeq 2.5$. ESR studies of solid glycine⁴⁰ and alanine⁴¹ at 90°K show the presence of the electron adduct radical $\text{NH}_3^+\text{CH}(\text{R})\dot{\text{C}}\text{OO}^-$ which dissociates on warming to yield $\text{NH}_3 + \dot{\text{C}}\text{H}(\text{R})\text{COO}^-$. The similarities between the radiation chemistry of these amino acids in the solid state and in aqueous solution are quite striking.

Higher molecular-weight amino acids also yield free ammonia as a major product on radiolysis in the evacuated solid state. Ammonia yields for solid aspartic acid, serine, phenylalanine, cystine and cysteine, for example, are all in the range $G \sim 2$ to $G \sim 5$.^{21,22,42,44} Systematic studies of the yields of organic products from these more complex amino acids with the exception of cysteine as noted below do not appear to have been made to date. However, ESR studies have confirmed the importance of the reductive deamination reaction 19 in the radiolysis of a number of these higher molecular-weight amino acids.^{45,46} The ESR studies also show that the spin centers formed in the ionization step 18 and in the abstraction reaction 20 are not confined to the α -carbon position as is the case with glycine and alanine. The side-chain radicals (as noted in section 2) would

preferentially dimerize on dissolution of the irradiated solid in water to yield α, α' -diamino acids as major reaction products.

Although the radiation chemistry of cysteine in aqueous solution is confined exclusively to the SH group, this is not the case in the radiolysis of solid cysteine. Free ammonia in the solid state is produced as a major product with $G(\text{NH}_3) \simeq 1.8$. An "NH₂-free" fraction of organic products is produced with $G \simeq 1.2$; β mercapto propionic acid is a major component of this fraction. The major features of the chemistry are consistent with the reaction sequence⁴⁴

followed by the radical removal steps

to give cystine, alanine and β mercapto propionic acid as the major organic products.

4. Peptides in Aqueous Solution

Radiation chemical studies of amino acid derivatives in aqueous solution containing added second solutes preferentially reactive toward e_{aq}^- and OH, have shown that reductive deamination by e_{aq}^- is a characteristic reaction of compounds containing a carbonyl bond α to the amino group.¹⁶ For the general case

where X represents O^- , OH, OR, NHR etc. For example, in the radiolysis of aqueous glycylglycine^{16,47,48}

which steps are then followed by

to give $G(\text{ammonia}) \simeq 3.8$, $G(\text{acetylglycine}) \simeq 2.9$, $G(\text{diamino succinic}) \simeq 1.7$.⁴⁸

The radical products of reactions 30,31 have been observed in pulse radiolysis studies of a number of aqueous peptide systems.^{49,50}

Reaction 29 has also been observed in ESR studies of the reaction of electrons with peptides in aqueous glasses at low temperature.^{51,52}

Analogous chemistry has been observed with oligo peptide derivatives of more complex amino acids.⁵³ In these cases, as with the corresponding free amino acid, OH attack can also occur along the amino acid side-chain (cf sec.2).^{21,22,54} With peptides, both types of radicals i.e.,

preferentially dimerize to yield α,α' -diamino acid derivatives.

TIME-SCALED EXPERIMENT

Fracture Logs

(XBL 791-87)

There is a marked increase in chemistry as the concentration of the peptide is increased above 0.1M.⁵⁸ With N-acetylalanine the ammonia yield increases from $G \sim 0.5$ at 0.1M to a limiting value of $G(\text{NH}_3) \simeq 2.8$ in the concentration range above $\sim 2\text{M}$. This increase in $G(\text{NH}_3)$ is accompanied by the formation of propionic acid as a major reaction product. In 0.1M N-acetylalanine solution $G(\text{propionic}) \simeq 0.1$. In 2M solutions $G(\text{propionic})$ approaches a value of 2. Addition of second solutes to 2M N-acetylalanine solutions to quantitatively scavenge e_{aq}^- (and OH) has essentially no effect on the process involved in formation of the amide and fatty acid.⁵⁸ The possibility that the electrons in concentrated peptide solutions are scavenged via reaction 44 (see below) prior to their hydration has been considered but there are certain stoichiometric arguments against this which may or may not be valid.^{58,60} There is also some experimental evidence that excited molecules are involved in the radiolytic cleavage of the peptide main chain in concentrated aqueous solution.^{58,60,61} It appears that more work will be required before the mechanisms for cleavage of the peptide chain in concentrated solution are completely understood.

5. Peptide in the Solid State

Main-chain cleavage with formation of amide and fatty acid functions is also a major reaction in the radiolysis of peptides in the solid state.^{58,62} Preliminary studies of this reaction were made with the N-acylamino acids,^{59,62} but subsequent work showed main-chain cleavage to be a major reaction mode in the radiolysis of the polyamino acids as well.⁵⁸ For acetylglutamine, acetylalanine and polyalanine, which have been studied in greater detail, the yields of major products (measured after

hydrolysis correspond to $G(\text{amide}) \simeq 3$, $G(\text{fatty acid}) \simeq 2$, $G(\text{keto acid}) \simeq 1$, and $G(\text{diamino acid}) \simeq 1$.^{58,59,62} The major reaction stoichiometries are accounted for in terms of the formulations

where the radical products of equation 40 represent the long-lived free radicals observed in solid peptides by ESR spectroscopy.^{45,63} The dehydropeptide formed in 41 reacts with water on dissolution to form amide and keto acid

The yield for amide production has been determined for a series of aliphatic, aromatic and sulfur-containing amino acids in the N-acetyl form.⁵⁸ 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 reaction.

Since the yield for amide and fatty acid production in the radiolysis of N-acetylalanine in 2M solution (Section 4) is essentially the same as it is in the solid state, it seems reasonable to consider the possibility that a common reaction is involved. As noted above, ESR studies of the reactions of electrons with N-acetyl alanine and

N-acetylglycine in aqueous glasses at low temperature^{51,52} have provided evidence for the reductive "deamidation" of the peptide bond via reaction 44.

The analogous reaction sequence has been observed in ESR studies of N-acetyl glycine in the solid state.^{45,64}

The stoichiometry of equation 40 would then be in accord with the reaction sequence

followed by the dimerization reaction 39. Supporting physical evidence of main-chain cleavage in the radiolysis of solid polyamino acids has been reported; in accord with the chemical findings, the irradiated samples show lower intrinsic viscosities and lower number average molecular weights.^{65,66}

The long-lived radical centers formed in steps 43, 45 may be located on the peptide main-chain (type I) as formulated above and/or on the side chain (type II) in the case of the more complex amino acid residues.^{45,46,63} In either case subsequent dimerization yields α, α' -diamino acid derivatives.

6. Enzymes and Proteins in Aqueous Solution

Early chemical studies⁶⁷⁻⁷¹ of the reactions of ionizing radiation on proteins in dilute aqueous solution were confined primarily to measurements of amino acid loss at relatively high dosages. These studies provided preliminary and qualitative evidence that the aromatic and heterocyclic residues and the cysteine-cysteine moieties are most susceptible to the indirect actions of ionizing radiation on proteins in oxygen-free

solution. The importance of C-H bonds of the peptide main-chain as major loci of OH attack in the radiolysis of structural proteins was established chemically some time later.⁷²

The pulse radiolysis-spectrophotometric technique is being used effectively in quantitative studies of the relative and absolute rates of reaction of e_{aq}^- and OH with the more reactive amino acid residues of protein. Such studies have provided specific quantitative information on the reaction of e_{aq}^- with various globular proteins at the disulfide linkage,⁷³⁻⁷⁵ at the unsaturated side chains of histidine and tryptophan, and at the carbonyl group of the peptide main chain.^{77,78} Similar studies have been made of the reactions of the OH radical with proteins at the SH linkage of cysteine,^{78,79} the unsaturated double bonds of histidine^{80,81} and the aromatic amino acids,^{82,83} and the C-H bonds of the peptide chain.⁷⁸ The use of selective free-radicals formed in the reactions $OH + 2CNS^- \rightarrow (CNS)_2^- + OH^-$; $OH + 2Br_2^- \rightarrow Br_2^- + OH^-$, has provided a very important pulse-radiolysis technique for the identification of specific amino acid residues essential to the activity of a particular enzyme system.⁷⁹⁻⁸³

Although there has been a great deal of very significant information obtained in these pulse radiolysis studies of the reactions of e_{aq}^- and OH with proteins in oxygen-free solution, still, our knowledge of the chemical nature and yields of the final organic products of these reactions is extremely limited. However, from the chemical studies made on model peptide systems (Sec. 1-4) it seems clear that radicals formed by OH attack at the peptide main-chain and at side chain loci yield radicals which in almost all cases preferentially dimerize to yield α, α' -diamino acid derivatives. We have also observed that the yield of these high

molecular weight dimers depends to a certain extent on the fate of the hydrated electron. If e_{aq}^- is captured by a non dissociative process at an unsaturated side-chain locus via reaction akin to 9 or at a peptide C=O linkage via reaction 35. Then reconstitution reactions as formulated in equations 10,37 can occur and will decrease the α,α' -diamino acid yield. On the other, dissociative capture of e_{aq}^- by an N-terminal C=O bond (eq. 29) or by a -SH linkage (eq. 23) for example, will lead to a maximal yield of α,α' -diamino acid derivatives. In any event, the main point here is that α,α' -diamino acids are major potential products of the radiolysis of proteins in aqueous media. It should be emphasized here also that the α,α' -diamino acids represent a class of compounds which (with the exception of cystine as noted in Sec. 2) are not found naturally in food protein. Such compounds have, however, been isolated from various bacteria and from certain pathogenic toxins.⁸⁴ In the following section we see that it is very likely that the α,α' -diamino acids are formed in even higher yield in the radiolysis of solid protein.

7. Enzymes and Proteins in the Solid State

Amino-acid analysis of both globular and fibrous proteins following irradiation in the evacuated solid state indicate that the various amino acids are destroyed more or less at random.⁸⁵⁻⁸⁸ These measurements of amino acid destruction are fairly approximate since dosages of 100 Mrad and above are required to produce a measurable decrease in the percent composition of a particular amino acid. These studies do show, however, that there is no highly preferential destruction of a relatively few amino acids as is observed in the aqueous case. The maximum variations in

"radiation sensitivity" in the radiolysis of solid proteins range over a factor of ~ 3 .

As observed with solid peptides (Sec. 5), the major degradation products formed in the radiolysis of solid proteins, both globular and fibrous, include: amide with $G \sim 2.5$,⁸⁶⁻⁸⁹ carbonyl (keto acid plus aldehyde) with $G \sim 1$,⁸⁶ fatty acids with $G \sim 1$,⁹⁰ and long-lived free radicals with $G \sim 5$.^{45,91} All of these chemical findings, together, strongly support the idea that the reaction stoichiometries represented by equations 40,41 for the polyamino acids are also of major importance in the radiolysis of solid proteins. Similarly, the evidence given in Sec. 5 for the ionic processes 43-45 as intermediate steps in the radiation "deamidation" of the main chain in peptides would appear to be equally valid in the radiolysis of solid proteins. In the protein case, the equivalent of the radical dimerization step 39 would yield a very complex mixture of symmetrical and unsymmetrical α, α' -diamino acid derivatives. The presence of heavy-metal ions (Cu^{+2} , Fe^{+2} , Ni^{+2}) exerts a pronounced protective effect on the enzymatic activity of irradiated solid enzymes.⁹²⁻⁹⁴ The presence of heavy-metal ions reduced the yield of stable free radicals observed at room temperature and also reduced the yield of main-chain cleavage in the radiolysis of solid fibrous proteins. These findings are consistent with the idea that the heavy-metal ions scavenge electrons, in competition with reaction 44.

Although the formation of amide and fatty acid functions in accordance with equation 40 explicitly states that cleavage of the peptide main chain occurs, this does not necessarily mean that lower molecular weight products will be observed. The average number molecular weight

of solid polyamino acids and fibrous proteins does indeed decrease on irradiation.^{66,91,95} However, globular proteins show a much lower yield of molecular fragments even after chemical reduction of intramolecular disulfide bonds.⁹⁶⁻¹⁰⁰ The reason for this difference can be related to the fact that in the irradiation of protein and high molecular weight polypeptides a number of main chain breaks plus the concomitant radical pair would be introduced into the macromolecular via equation 40 even at the lowest practicable dosages. On the dissolution of irradiated globular proteins, radical combination within the glob would be favored by the constraints imposed by the secondary and tertiary structures. With the polyamino acids and fibrous proteins such constraints are minimal and the separation of radical fragments on dissolution would be competitive with combination.^{21,22,91}

8. Summary and Conclusions

The present detailed evaluation of the various types of chemistry involved in the radiolysis of amino acids and peptides raises new questions regarding the wholesomeness of irradiated high-protein foods. Of particular concern is the fact that the radiolysis of peptide derivatives of the α -amino acids found in protein leads to synthesis of high molecular-weight α, α' -diamino acids as major products both in aqueous and solid systems. These α, α' -diamino acids represent a class of compounds not normally found in plant and animal protein sources. Such compounds have, however, been isolated from several bacteria and from certain pathogenic toxins.

Although no detailed chemical analyses for α, α' -diamino acids in irradiated protein have been undertaken, all of the chemical and physical

evidence available to date indicates that such compounds are produced in the radiolysis proteins both in aqueous and solid systems. Admittedly, the isolation and quantitative determination of α, α' -diamino acids in irradiated protein represent a formidable experimental undertaking because of the anticipated complexity of the mixture of diamino acids which could be produced in the dimerization of peptide radicals of types I and II. It seems imperative, however, that such a program be initiated to establish whether or not the radiation synthesis of α, α' -diamino acids in high protein foods is an important factor in wholesomeness considerations. This is particularly true because the present radiation chemical evidence tends to support the position of the U. S. Food and Drug Administration viz that ionizing radiation should be classified as a food additive.¹⁰¹

The successful application of radiation sterilization techniques to high protein foods such as meats requires, in most cases, that the food be in the frozen state at $-30^\circ \pm 10^\circ\text{C}$ during irradiation to obtain a product with acceptable taste and aroma.^{1,2,5} Meat irradiated in the frozen state will undergo less net chemical change per unit dose than that irradiated above 0°C .⁵ However, as the temperature is lowered below 0°C higher irradiation doses are required to achieve the same biocidal effect.¹ In frozen aqueous systems the recombination of primary radical and ion pairs is favored because diffusion processes are impeded in the solid.^{102,103} However, the fraction of e_{aq}^- and OH that can be chemically scavenged in a frozen solution is strongly dependent on solute concentration. With molar concentrations of solutes that are effective scavengers for both e_{aq}^- and OH, the observed chemical yields in frozen solutions at low temperature represent a major fraction of that observed in the corresponding liquid

system.^{103,104} Since the organic components of meat, on the basis of weight percent, correspond to an ~ 5 molar solution of reactive organic solute (MW=100), it is concluded that the chemistry induced by reactions of e_{aq}^- and OH in meat at -30°C can be quite significant. ESR measurements of radical yields in the radiolysis of solid proteins indicate that net chemistry arising from equation 40 at -30°C is about 80 to 90 percent of that observed at room temperature.^{105,106}

An explanation for the very great decrease in the yield of odor-causing products from meat irradiated at -30°C as compared to the yield at room temperature can be readily formulated. Assume that the precursor of an odor-causing product RH is the radical R and that the primary yield of R is not greatly dependent on temperature over the range 0° to -30°C . Two competing processes can be considered to be involved in the removal of R

Since the activation energy for dimerization is less than that for abstraction, reaction 47 would be favored at low temperature. The higher molecular-weight product R_2 would be less volatile (or non-volatile) and would contribute less to the odor of the irradiated product.

Acknowledgment

This work was supported by the Division of Chemical Sciences, Office of Basic Energy Sciences, U. S. Department of Energy under contract No. W-7405-ENG-48, and the U. S. Army Research-Battelle Scientific Project.

REFERENCES

1. E. J. Josephson, A. Brynjolfsson and E. Wierbicki, The Use of Ionizing Radiation in the Preservation of Food and Feed Products in Radiation Research: Biomedical, Chemical and Physical Perspectives, pg. 96, Academic Press, N.Y. 1975.
2. A. Brynjolfsson, High Dose and Low Dose Food Irradiation Programs in the United States of America in Food Preservation by Irradiation. Vol 7, pg. 15, IAEA, Vienna, 1978.
3. K. Vas, The Development of International Standards for Irradiated Foods, IAEA Bull. 20, 5 (1978).
4. E. S. Josephson, A. Brynjolfsson, E. Wierbicki, D. B. Rowley C. Merrit, R. W. Baker, J. J. Killoran and M. H. Thomas. Radappertization of Meat, Meat Products and Poultry in Radiation Preservation of Food, pg. 471 IAEA, Vienna (1973).
5. I. A. Taub, R. A. Kaprielian, and J. W. Halliday, Radiation Chemistry of High Protein Foods Irradiated at Low Temperatures in Food Preservation by Irradiation, Vol. I. pg. 371, IAEA, Vienna, 1978.
6. I. G. Draganic and Z. D. Draganic, The Radiation Chemistry of Water, Academic Press, N.Y. (1971).
7. J. W. T. Spinks and R. J. Woods, An Introduction to Radiation Chemistry, J. Wiley and Sons, N.Y. (1976).
8. G. Stein and J. Weiss, Chemical Actions of Ionizing Radiations on Aqueous Solutions. The Actions of X-rays on Some Amino-acids. J. Chem. Soc. 3756 (1949).

9. W. M. Dale, J. V. Davies and C. W. Gilbert, The Kinetics and Specificities of Deamination of Nitrogenous Compounds by X-irradiation, *Biochem. J.* 45, 93 (1949).
10. C. R. Maxwell, D. C. Peterson, and N. E. Sharpless, The Effect of Ionizing Radiation on Amino Acids. I. The Effect of X-rays on Aqueous Solutions of Glycine, *Radiat. Res.* 1, 530 (1954).
11. N. E. Sharpless, A. E. Blair, and C. R. Maxwell, The Effect of Ionizing Radiation on Amino Acids. II. The Effects of X-rays on Aqueous Solutions of Alanine, *Radiat. Res.* 2, 135 (1955).
12. B. M. Weeks and W. M. Garrison, Mechanism in the Radiolysis of Aqueous Solutions of Glycine, *Radiat. Res.* 9, 291 (1958).
13. W. M. Garrison, Actions of Ionizing Radiations on Nitrogenous Compounds in Aqueous Media, *Radiat. Res. Suppl.* 4, 158 (1964).
14. B. M. Weeks, S. Cole, and W. M. Garrison, Reactions of Alanine with the Reducing Species Formed in Water Radiolysis, *J. Phys. Chem.* 69, 4631 (1965).
15. R. L. S. Willix and W. M. Garrison, Effect of Cupric Ion on the Radiation Chemistry of Aqueous Glycine, *J. Phys. Chem.* 69, 1579 (1965).
16. R. L. S. Willix and W. M. Garrison, Chemistry of the Hydrated Electron in Oxygen-Free Solutions of Amino Acids, Peptides and Related Compounds, *Radiat. Res.* 32, 452 (1967).
17. P. Neta, M. Simic, and E. Hayon, Pulse Radiolysis of Aliphatic Acids in Aqueous Solution. III. Simple Amino Acids, *J. Phys. Chem.* 74, 1214 (1970).

18. M. D. Sevilla, Radicals Formed by the Reaction of Electrons with Amino Acids in an Alkaline Glass, J. Phys. Chem. 74, 2096 (1970).
19. J. Kopoldova, J. Liebster, and A. Babicky, The Mechanism of the Radiation-Chemical Degradation of α -Aminobutyric Acid in Oxygen-free Solution, Int. J. Appl. Rad. Isotopes 13, 617 (1962).
20. J. Kopoldova, J. Liebster, and A. Babicky, The Mechanism of the Radiation - Chemical Degradation of α -Aminobutyric Acid in Oxygenated Solution, Int. J. Appl. Rad. Isotopes, 11, 139 (1961).
21. W. M. Garrison, Radiation Chemistry of Organo-Nitrogen Compounds, Curr. Top. Radiat. Res. 4, 43 (1968).
22. W. M. Garrison, Radiation Induced Reactions of Amino Acids and Peptides, Radiat. Res. Rev. 3, 305 (1972).
23. G. A. Brodskaya and V. A. Sharpatyi, Radiolysis of Aqueous Solutions of Phenylalanine, Russ. J. Phys. Chem. (English trans.) 41, 583 (1967).
24. G. A. Brodskaya and V. A. Sharptyi, The Concentration Effect in the Radiolysis of Aqueous Phenylalanine, Russ. J. Phys. Chem. (English Trans.) 43, 1343 (1969).
25. J. Mittal and E. Hayon, Interaction of Hydrated Electrons with Phenylalanine and Related Compounds, J. Phys. Chem. 78, 1790 (1974).
26. J. Nosworthy and C. B. Allsopp, Effects of X-rays on Dilute Aqueous Solutions of Amino Acids, J. Colloid Sci. 11, 565 (1956).
27. J. Chrysochoos, Pulse Radiolysis of Phenylalanine and Tyrosine, Radiat. Res. 33, 465 (1968).

38. G. E. Adams, G. S. McNaughton, and D. B. Michael, The Pulse Radiolysis of Sulfur Compounds. Part I. Cysteamine and Cystamine. in The Chemistry of Ionization and Excitation, pg. 281, G. R. A. Johnson and G. Scholes, Eds. (1967).
39. M. Hoffman and E. Hayon, Pulse Radiolysis Study of Sulfhydryl Compounds in Aqueous Solution, J. Phys. Chem. 77, 990 (1973).
40. H. C. Box, H. G. Freund, and E. E. Budzinski, Paramagnetic Absorption of Irradiated Glycine, J. Am. Chem. Soc. 88, 658 (1966).
41. J. W. Sinclair and M. W. Hanna, Electron Paramagnetic Resonance Study of L-Alanine Irradiated at Low Temperatures, J. Phys. Chem. 71, 84 (1967).
42. V. G. Peter and B. Rajewsky, The Action of Ionizing Radiation on Amino Acids, Z. Naturforschg. 18b, 110 (1963).
43. K. Dose and B. Radjewsky, The Chemistry of Direct and Indirect Radiation Effects on Amino Acids, Biochem. Z. 330, 131 (1958).
44. D. B. Peterson, J. Holian, and W. M. Garrison, Radiation Chemistry of the α -Amino Acids. γ -Radiolysis of Solid Cysteine, J. Phys. Chem. 73, 1568 (1969).
45. T. Henriksen, T. B. Melø, and G. Saxebøl, Free Radical Formation in Proteins (and Peptides) and Protection from Radiation Damage in Free Radicals in Biology, Vol II, Acad. Press, (1976).
46. L. S. Myers, Jr., Radiation Chemistry of Nucleic Acids, Proteins, and Polysaccharides, in The Radiation Chemistry of Macromolecules, M. Dole, Ed., Academic Press, N.Y. (1973).

38. G. E. Adams, W. S. McNaughton, and D. B. Michael, The Pulse Radiolysis of Sulfur Compounds. Part I. Cysteamine and Cystamine. in The Chemistry of Ionization and Excitation, pg. 281, G. R. A. Johnson and G. Scholes, Eds. (1967).
39. M. Hoffman and E. Hayon, Pulse Radiolysis Study of Sulphydryl Compounds in Aqueous Solution, J. Phys. Chem. 77, 990 (1973).
40. H. C. Box, H. G. Freund, and E. E. Budzinski, Paramagnetic Absorption of Irradiated Glycine, J. Am. Chem. Soc. 88, 658 (1966).
41. J. W. Sinclair and M. W. Hanna, Electron Paramagnetic Resonance Study of L-Alanine Irradiated at Low Temperatures, J. Phys. Chem. 71, 84 (1967).
42. V. G. Peter and B. Rajewsky, The Action of Ionizing Radiation on Amino Acids, Z. Naturforschg. 18b, 110 (1963).
43. K. Dose and B. Radjewsky, The Chemistry of Direct and Indirect Radiation Effects on Amino Acids, Biochem. Z. 330, 131 (1958).
44. D. B. Peterson, J. Holian, and W. M. Garrison, Radiation Chemistry of the α -Amino Acids. γ -Radiolysis of Solid Cysteine, J. Phys. Chem. 73, 1568 (1969).
45. T. Henriksen, T. B. Melø, and G. Saxebøl, Free Radical Formation in Proteins (and Peptides) and Protection from Radiation Damage in Free Radicals in Biology, Vol II, Acad. Press, (1976).
46. L. S. Myers, Jr., Radiation Chemistry of Nucleic Acids, Proteins, and Polysaccharides, in The Radiation Chemistry of Macromolecules, M. Dole, Ed., Academic Press, N.Y. (1973).

47. W. Bennett-Corniea, H. A. Sokol and W. M. Garrison, Reductive Deamination in the Radiolysis of Oligopeptide in Aqueous Solution and in the Solid State, *Radiat. Res.* 43, 257 (1970).
48. W. M. Garrison, H. A. Sokol, and W. Bennett-Corniea, Radiation Chemistry of Glycylglycine in Oxygen-Free Systems, *Radiat. Res.* 53, 376 (1973).
49. M. Simic and E. Hayon, Reductive Deamination of Oligopeptides by Solvated Electrons in Aqueous Solution, *Radiat. Res.* 48, 244 (1971).
50. P. S. Rao and E. Hayon, Reactions of Hydroxyl Radicals with Oligopeptides in Aqueous Solution. A Pulse Radiolysis Study, *J. Phys. Chem.* 79, 109 (1975).
51. M. D. Sevilla, Radicals Formed by Electron Attachment to Peptides, *J. Phys. Chem.* 74, 366 (1970).
52. M. D. Sevilla and L. V. Brooks, Radicals Formed by the Reactions of Electrons with Amino Acids and Peptides in a Neutral Aqueous Glass, *J. Phys. Chem.* 77, 2954 (1973).
53. J. Liebster and J. Kopoldova, Radiation Chemical Reactions in Aqueous Oxygenated and Oxygen-Free Solutions of Aliphatic Dipeptides and Tripeptides, *Radiat. Res.* 27, 162 (1966).
54. H. Sokol, W. Bennett-Corniea and W. M. Garrison, A Marked Effect of Conformation in the Radiolysis of Poly- α -L-glutamic Acid in Aqueous Solution, *J. Am. Chem. Soc.* 87, 1391 (1965).
55. M. Farragi and Y. Tal, Reaction of Hydrated Electrons with Amino Acids, Peptides and Proteins in Aqueous Solution. II. Formation of Radicals and Electron Transfer Reactions, *Radiat. Res.* 62, 347 (1975).

56. J. Holian, and W. M. Garrison, On the Radiation-Induced Reduction of Amide and Peptide Functions in Aquoorganic Systems, J. Phys. Chem. 72, 4721 (1968).
57. P. S. Rao and E. Hayon Interaction of Hydrated Electron with the Peptide Linkage, J. Phys. Chem. 78, 1193 (1974).
58. W. M. Garrison et al., Ionization and Excitation in Peptide Radiolysis, Advances in Chemistry Series 81, "Radiation Chemistry" II, 384 (1968).
59. W. M. Garrison and B. M. Weeks, Radiation Chemistry of Compounds Containing the Peptide Bond, Radiat. Res. 17, 341 (1962).
60. W. M. Garrison and M. A. J. Rogers, Role of Excited Species $(\text{RCO-NH-CHR}_2)^*$ in the Radiolytic Oxidation of the Peptide Main-Chain in Aqueous Systems, J. Radiat. Phys. Chem. 1, 541 (1969).
61. M. A. J. Rodgers, H. A. Sokol, and W. M. Garrison, Radiolytic Cleavage of the Peptide Main-Chain in Concentrated Aqueous Solution: Energy Level of Excited-Molecule Intermediates, Biochem. Biophys. Res. Comm. 40 622 (1970).
62. W. M. Garrison, M. E. Jayko, B. M. Weeks, H. A. Sokol, and W. Bennett-Corniea, Chemical Evidence for Main-Chain Scission as a Major Decomposition Mode in the Radiolysis of Solid Peptides, J. Phys. Chem. 71, 1546 (1967).
63. R. C. Drew and W. Gordy, Electron Spin Resonance Studies of Radiation Effects on Polyamino Acids, Radiat. Res. 18, 552 (1963).

64. G. Saxebøl, An ESR Study of Free Radical Conversions in Irradiated Single Crystals of N-Acetylglycine in the Temperature Range 77-300 K, *Int. J. Radiat. Biol.* 24, 475 (1973).
65. M. Kunikane and S. Sugai, Effects of γ -rays on Poly-L-Glutamic Acid and Poly-D, L-Glutamic Acid in the Solid State, *Biophysik* 7, 205 (1971).
66. G. A. Hayden, S. C. Rodgers, and F. Friedberg, Radiation Degradation of Polyamino Acids in the Solid State, *Arch. Biochem. Biophys.* 113, 247 (1966).
67. E. S. G. Barron, J. Ambrose and P. Johnson, Studies on the Mechanisms of Action of Ionizing Radiations VIII The Effect of X-Irradiation on Some Physicochemical Properties of Proteins, *Radiat. Res.* 2, 145 (1955).
68. M. P. Drake, J. W. Giffe, D. A. Johnson and V. L. Koenig, Chemical Effects of Ionizing Radiation on Proteins, I Effect of γ -Radiation on the Amino Acid Content of Insulin, *J. Am. Chem. Soc.* 79, 1395 (1957).
69. S. Okada and G. Gehrmann, Inactivation of Desoxyribonucleic Acid by X-rays, *Biochim. et. Biophys. Acta* 25, 179 (1957).
70. R. Lange and A. Pihl, The Mechanism of X-ray Inactivation of Phosphoglyceraldehyde Dehydrogenase, *Intern. J. Radiat. Biol.* 2, 301 (1960).
71. R. J. Romani and A. L. Tappel, Anaerobic Irradiation of Alcohol Dehydrogenase, Aldose, and Ribonuclease, *Arch. Biochem. Biophys.* 79, 323 (1959).

72. W. M. Garrison, M. E. Jayko and W. Bennett, Radiation Induced Oxidation of Protein in Aqueous Solution, Radiat. Res. 16 483 (1962).
73. G. M. Gaucher, B. L. Mainman, G. P. Thompson, and D. A. Armstrong, The Co 60 -Radiolysis of Aqueous Papsin Solutions Repair and Protection by Cysteine, Radiat. Res. 46 (3) 456 (1971).
74. M. Lal, W. S. Liu, G. M. Gaucher, and D. A. Armstrong, Repair, Protection, and Sensitization of Papain with Respect to Inactivation by H_2O_2 and OH .
75. G. E. Adams, K. F. Baverstock, R. B. Cundall and J. L. Redpath, Radiation Effects on α -chymotrypsin in Aqueous Solutions: Pulse Radiolysis and Inactivation Studies, Radiat. Res. 54, 375 (1973).
76. R. H. Bisby, R. B. Cundall, J. L. Redpath, and G. E. Adams, One-Electron Reactions with Enzymes in Solution: A Pulse Radiolysis Study, J. Chem. Soc. Faraday Trans. I. 72, 51 (1976).
77. W. S. Lind, J. R. Slement, G. M. Gaucher, and D. A. Armstrong, Repairable and Non-Repairable Inactivation of Irradiated Aqueous Papain. Effects of OH , O_2^- , e_{aq}^- and H_2O_2 , Radiat. Res. 62, 438 (1975).
78. N. N. Lichtin, et al., Fast Consecutive Radical Processes within Ribonuclease Molecule In Aqueous Solution. II. Reaction with OH and e_{aq}^- , Biochim. Biophys. Acta. 276, 124 (1972).
79. G. E. Adams and J. L. Redpath, Selective Free-Radical Reactions with Protein and Enzymes: Pulse Radiolysis and Interactivation Studies On Papain, Int. J. Radiat. Biol. 25, (2) 129 (1974).

80. K. Baverstock, R. B. Cundall, G. E. Adams, and J. L. Redpath,
Selective Free-Radical Reactions with Proteins and Enzymes. The
Inactivation of α -chymotrypsin. *Int. J. Radiat. Biol.* 26, 39 (1974).
81. J. L. Redpath, et al., Role of Metal Ions in the Radiosensitivity
of Metalloproteins. Model Experiments with Bovine Carbonic Anhydrase.
Int. J. Radiat. Biol. 28, 243 (1975).
82. J. E. Aldrich, R. B. Cundall, G. E. Adams, and R. L. Willson,
Identification of Essential Residues in Lysozyme: A Pulse Radiolysis
Method. *Nature* 221, 1049 (1969).
83. G. E. Adams, R. H. Bisby, R. B. Cundall, J. L. Redpath, and
R. L. Willson, Selective Free Radical Reactions with Proteins and
Enzymes. The Inactivation of Ribonuclease. *Radiat. Res.* 49, 290
(1972).
84. J. P. Greenstein and M. Winitz, Chemistry of the Amino Acids,
J. Wiley and Sons, N.Y. (1961).
85. A. Caputo and K. Dose, Direct Action of X-Rays on Proteins, Peptides,
and Amino Acids. *Z. Naturforschung* 12b, 172 (1957).
86. P. Alexander and L. D. G. Hamilton, Irradiation of Proteins in the
Solid State. Part II. Chemical Changes Produced in Bovine Serum
Albumin. *Radiat. Res.* 13, 214 (1960).
87. J. H. Bowes and J. A. Moss, The Effect of γ -Radiation on Collagen.
Radiat. Res. 16, 211 (1962).
88. M. Burke and L. Augenstein, A Comparison of the Effects of Ultra-
violet and Ionizing Radiations on Trypsin Activity and on Its
Constituent Amino Acids. *Biochem. J.* 114, 535 (1969).

89. D. R. Cooper and A. E. Russell, Decomposition of Soluble Collagen by γ -Irradiation. *Biochem. J.* 113, 263 (1969).
90. F. Freidberg and P. Riesz, Acetic and Propionic Acid Productions From γ -Irradiated Dry Proteins. *Radiat. Res.* 42, 446 (1970).
91. F. Friedberg, The Effect of Ionizing Radiation on Solid Proteins. *Radiat. Res. Rev.* 2, 131 (1969).
92. P. Riesz, The Role of the Metal Ions in the γ -Radiolysis of Dry Metal-Ribonuclease Complexes. *Biochem. Biophys. Res. Commun.* 23, 273 (1966).
93. B. B. Singh and M. G. Omerrod, The Effect of Metal Ions on Free Radical Formation and Reactions in Irradiated Proteins (Solid). *Int. J. Radiat. Biol.* 10, 369 (1966).
94. F. Friedberg, S. Kominami, A. K. N. Nandekar, and P. Riesz, Effect of Metal Ions on the Formation of Chain Breaks and Radicals in γ -Irradiated Collagen and Polyadenylic Acid. *Radiat. Res.* 61, 55 (1975).
95. F. Friedberg, Effect of Irradiation on Some Lyophilized Proteins. *Radiat. Res.* 38, 34 (1969).
96. J. S. Haskill and J. W. Hunt, Radiation Damage to Crystalline Ribonuclease. Identification of the Physical Alterations by Gell Filtration on Sephadex. *Radiat. Res.* 31, 327 (1967).
97. J. S. Haskill and J. W. Hunt, Radiation Damage to Crystalline Ribonuclease Importance of Free Radicals in the Formation of Denatured and Aggregated Products. *Radiat. Res.* 32, 606 (1967).
98. J. S. Haskill and J. W. Hunt, Radiation Damage to Crystalline RNase: Identification of Polypeptide Chain Breakage in Denatured and Aggregated Products. *Radiat. Res.* 32, 827 (1967).

99. S. M. Herbert and B. M. Tolbert, Chemical and Conformational Studies of Gamma-Irradiated Solid Lysozyme. *Radiat. Res.* 65, 268 (1976).
100. D. J. Marciani and B. M. Tolbert, Analytical Studies of Fractions from Irradiated Lysozyme. *Biochem. Biophys. Acta.* 271, 262 (1972).
101. Federal Food, Drug and Cosmetic Act, as amended, 21 U.S. Code 321, 21 Code of Federal Regulations, Part 121-Food Additives (1958).
102. L. Kevan, Radiation Chemistry of Frozen Aqueous Solutions, in Radiation Chemistry of Aqueous Solutions, G. Stein, Ed., J. Wiley and Sons, N.Y. (1968).
103. B. G. Ershov and A. K. Pikaev, Stabilized Free Radicals in the Radiation Chemistry of Frozen Aqueous Solutions. *Radiat. Res. Rev.* 21, (1969).
104. K. Kishore, P. N. Moorthy and K. N. Rao, Radiation Chemistry of Nitrate Ices. *Radiat. Phys. Chem.* 11, 239 (1978).
105. T. Henriksen, T. Sanner and A. Pihl, Secondary Processes in Proteins Irradiated in the Dry State. *Radiat. Res.*, 18, 147 (1963).
106. T. Henriksen, Effect of Irradiation Temperature on the Production of Free Radicals in Some Biological Compounds Exposed to Various Ionizing Radiations. *Radiat. Res.*, 27, 694 (1966).