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

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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 bacterial products. 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.

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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% respectively. Radiation chemical change in the protein and lipid fractions arises both 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

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

where e^-_{aq} represents the hydrated electron. For gamma rays and fast electrons the 100 eV yields, (G), of the free radical products correspond to $G(OH) \cong 2.8$, $G(e^-_{aq}) \cong 2.7$, $H(H) \cong 0.55$.

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 the formulation of the reaction scheme[12-16]

$$e^-_{aq} + NH_3^+CH(R)COO^- \rightarrow NH_3 + \dot{C}H(R)COO^- \qquad (2)$$

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

followed by

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

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

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

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

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

The overall stoichiometry of reactions 2-7 gives

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

The yield of higher molecular-weight products from glycine and alanine is low. Radicals of the type $NH_3^+\dot{C}(R)COO^-$ disproportionate almost quantitatively as shown in steps 5,6. In the case of glycine a small fraction of the $NH_3^+\dot{C}HCOO^-$ radicals undergoes dimerization to yield α,α'-diaminosuccinic acid[12]

$$COOH - CH_2(NH_2) - CH_2(NH_2) - COOH$$

Since neither ethylamine nor β-alanine were found to undergo the reductive deamination reaction 2, it was proposed[14-16] that e^-_{aq} adds to the C=O bond of the α-amino acid and that the reduced intermediate then dissociates.

$$e^-_{aq} + NH_3^+CH(R)COO^- \rightarrow NH_3^+CH(R)C\cdot\langle^{O^-}_{O} \longrightarrow NH_3 + \dot{C}H(R)COO^- \qquad (2a)$$

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, for example, both α,α'-diamino suberic acid

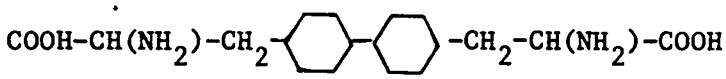
$$COOH\text{-}CH(NH_2)\text{-}(CH_2)_4\text{-}CH(NH_2)\text{-}COOH$$

and α,α'-diaminomethyl pimelic acid

$$COOH\text{-}CH(NH_2)\text{-}(CH_2)_2\text{-}CH(CH_3)\text{-}CH(NH_2)\text{-}COOH$$

are formed as major products with a combined yield of $G \simeq 3$.[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) $\simeq 5$.[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 $G(OH) + G(e^-_{aq}) \simeq 5$. 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]

$$OH + M \rightarrow \dot{M}OH \qquad (8)$$

$$e^-_{aq} + M \rightarrow \dot{M}H + OH^- \qquad (9)$$

$$\dot{M}OH + \dot{M}H \rightarrow M + M(HOH) \qquad (10)$$

$$M(HOH) \rightarrow M + H_2O \qquad (11)$$

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]

$$OH + RH \rightarrow R \quad (12)$$

$$\dot{M}H + R \rightarrow MH(R) \quad (13)$$

The radiation chemistry of cysteine ($NH_3^+CH(CH_2SH)COO^-$) and other aliphatic thiols in dilute oxygen free solution occurs exlusively at the SH group[36,37]

$$e^-_{aq} + RSH \rightarrow R + HS^-(H_2S) \quad (14)$$

$$OH + RSH \rightarrow RS + H_2) \quad (15)$$

followed by

$$R + RSH \rightarrow RH + RS \quad (16)$$

$$2RS \rightarrow RSSR \quad (17)$$

to give G(cystine) $\simeq$ G(alanine) + G(H_2S) $\simeq$ 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.

$$COOH - CH(NH_2) - CH_2\text{-}S\text{-}S\text{-}CH_2 - CH(NH_2) - COOH$$

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 reacion sequence:[13,21,22]

$$NH_3^+CH(R)COO^- \rightsquigarrow NH_3^+\dot{C}(R)COO^- + H^+ + e^- \quad (18)$$

$$e^- + NH_3^+CH(R)COO^- \rightsquigarrow NH_3^+CH(R)\dot{C}OO^= \rightarrow NH_3 + \dot{C}H(R)COO^- \quad (19)$$

followed by:

$$\dot{C}H(R)COO^- + NH_3^+CH(R)COO^- \rightarrow {\cdot}CH_2(R)COO^- + NH_3^+\dot{C}(R)COO^- \qquad (20)$$

$$2NH_3^+\dot{C}(R)COO^- \rightarrow NH_2^+{=}C(R)COO^- + NH_2^+CH(R)COO^- \qquad (21)$$

and

$$H_2O + NH_2^+{=}C(R)COO^- \rightarrow NH_4^+ + RCOCOO^- \qquad (22)$$

on dissolution of the irradiated solid in O_2-free water. With glycine and alanine at room temperature: $G(NH_3) \simeq 5$, $G(RCOCOOH) \simeq 2.5$, $G(RCH_2COOH) \simeq 2.5$. ESR studies of solid glycine[40] and alanine[41] at 90°K show the presence of the electron adduct radical $NH_3^+CH(R)\dot{C}OO^=$ which dissociates on warming to yield $NH_3 + \dot{C}H(R)COO^-$. The similarities between the radiation chemistry of these 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 preferntially 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 $G(NH_3) \simeq 1.8$. An "NH_2-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 sequence[44]

$$RSH \rightsquigarrow R\dot{S} + H^+ + e^- \quad (23)$$

$$e^- + RSH \rightarrow NH_3 + \dot{C}H(CH_2SH)COO^- \quad (24)$$

$$e^- + RSH \rightarrow H_2S + NH_2CH(\dot{C}H_2)COO^- \quad (25)$$

followed by the radical removal steps

$$RSH + \dot{C}H(CH_2SH)COO^- \rightarrow R\dot{S} + CH_2(CH_2SH)COO^- \quad (26)$$

$$RSH + NH_2CH(\dot{C}H_2)COO^- \rightarrow R\dot{S} + NH_2CH(CH_3)COO^- \quad (27)$$

$$2RS \rightarrow RSSR \quad (28)$$

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

$$e_{aq}^- + NH_3^+C(R_2)C\begin{smallmatrix}\nearrow O\\ \searrow X\end{smallmatrix} \rightarrow NH_3^+C(R_2)C\cdot\begin{smallmatrix}\nearrow O^-\\ \searrow X\end{smallmatrix} \rightarrow NH_3 + \dot{C}R_2C\begin{smallmatrix}\nearrow O\\ \searrow X\end{smallmatrix} \quad (29)$$

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

$$e_{aq}^- + NH_3^+CH(R)CONHCH(R)COO^- \rightarrow NH_3 + \dot{C}H(R)CONHCH(R)COO^- \quad (30)$$

$$OH + NH_3^+CH(R)CONHCH(R)COO^- \rightarrow H_2O + NH_3^+CH(R)CNH\dot{C}(R)COO^- \quad (31)$$

which steps are then followed by

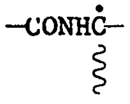
$$\dot{C}H(R)CONHCH(R)COO^- + NH_3^+CH(R)CONHCH(R)COO^- \rightarrow$$
$$CH_2(R)CONHCH(R)COO^- + NH_3^+CH(R)CONH\dot{C}(R)COO^- \quad (32)$$

$$2NH_3^+CH(R)CONH\dot{C}(R)COO^- \rightarrow \begin{matrix}NH_3^+CH(R)CONHC(R)COO^-\\ |\\ NH_3^+CH(R)CONHC(R)COO^-\end{matrix} \quad (33)$$

to give G(ammonia) $\simeq$ 3.8, G(acetylglycine) $\simeq$ 2.9, G(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.,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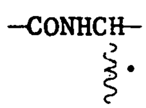
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preferentially dimerize to yield α,α'-diamino acid derivatives.

As the chain length of the oligopeptide is increased, peptide C=O bonds other than those of the N-terminal amino acid become involved as trapping centers for e^-_{aq} i.e.

$$e^-_{aq} + NH_3^+CH_2CONHCH_2CONHCH_2COO^- \rightarrow NH_3^+CH_2CONHCH_2\dot{C}(O^-)NHCH_2COO^- \quad (34)$$

There is evidence that electrons trapped along the peptide chain via reaction 20 can migrate to the C=O group of the N-terminal amino acid through an intermolecular process.[55]

In the radiolysis of N-acetyl and polyamino acid derivatives of glycine and alanine, e^-_{aq} adds essentially quantitatively to the peptide C=O bond i.e.,[56,57]

$$e^-_{aq} + RCONHCHR_2 \rightarrow R\dot{C}(OH)NHCHR_2 + OH^- \quad (35)$$

$$OH + RCONHCHR_2 \rightarrow RCONH\dot{C}R_2 + H_2O \quad (36)$$

There is little net chemical change as a consequence of reactions 35,36 since the reconstruction reaction

$$R\dot{C}(OH)NHCHR_2 + RCONH\dot{C}R_2 \rightarrow 2RCONHCHR_2 \quad (37)$$

represents the major path for radical removal.[58] However, in the presence of a second solute which converts e^-_{aq} to an abstracting radical e.q. $e^-_{aq} + H^+ \rightarrow H$, $e^-_{aq} + RX \rightarrow R + X^-$, then

$$RCONHCHR_2 + H(R) \rightarrow RCONH\dot{C}R_2 + H_2(RH) \quad (38)$$

and the peptide radicals formed via steps 36, 38 dimerize to yield the α,α'-diamino acid with $G \simeq 3$.[59]

$$2RCONH\dot{C}R_2 \rightarrow \begin{array}{c} RCONHCR_2 \\ | \\ RCONHCR_2 \end{array} \quad (39)$$

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 $G(NH_3) \simeq 2.8$ in the concentration range above $\sim$ 2M. This increase in $G(NH_3)$ is accompanied by the formation of propionic acid as a major reaction product. In 0.1M N-acetylalanine solution G(propionic) $\simeq$ 0.1. In 2M solutions G(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 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 acetylglycine, acetylalanine and polyalanine, which have been studied in greater detail, the yields of major products (measured after hydrolysis correspond to G(amide $\simeq$ 3. G(fatty acid) $\simeq$ 2, G(keto acid) $\simeq$ 1, and G(diamino acid) $\simeq$ 1.[58,59,62] The major reaction stoichiometries are accounted for in terms of the formulations

$$3RCONHCHR_2 \rightarrow RCONH_2 + CH_2R_2 + 2RCONH\dot{C}R_2 \quad (40)$$

$$RCONHCHR_2 \rightarrow RCON{=}CR_2 + H_2 \quad (41)$$

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

$$H_2O + RCON{=}CR_2 \rightarrow RCONH_2 + R_2CO \quad (42)$$

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

$$RCONHCHR_2 \rightsquigarrow RCONH\dot{C}R_2 + H^+ + e^- \quad (43)$$

$$e^- + RCONHCHR_2 \rightarrow R\overset{O^-}{\overset{|}{C}}NHCHR_2 \rightarrow RCONH^- + \dot{C}HR_2 \quad (44)$$

$$\dot{C}HR_2 + RCONHCHR_2 \rightarrow RCONH\dot{C}R_2 + CH_2R_2 \quad (45)$$

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 1) 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 studies[67-71] 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-cystine 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 seen 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, by isolated from various bacteria and from certain bacterial products.[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 a 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 ~2.5,[86-89] carbonyl (keto acid plus aldehyde) with G ~ 1,[86] fatty acids with G ~1,[90] and long-lived free radicals with G ≃ 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 appeqr 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. Temperature Effects

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° ± 10°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 sold.[101,107] 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.[5,102,103] 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.[104,105]

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.[105] Two competing processes can be considered to be involved in the removal of R

$$R + R'H \rightarrow RH + R' \qquad (46)$$

$$2R \rightarrow R_2 \qquad (47)$$

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.

9. 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 biochemistry of irradiated high-protein foods. Of particular interest 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 bacterial products.

Although no detailed chemical analyses for α,α'-diamino acids in irradiated protein have been undetaken, 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 important, however, that such a program be initated 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]

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