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Author
Garrison, W.M.

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CHEMICAL ACTIONS OF IONIZING RADIATION ON OLIGOPEPTIDE DERIVATIVES OF GLYCINE IN THE NEUTRAL (ZWITTERION) AND BASIC FORMS

Warren M. Garrison and Harvey A. Sokol

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Chemical Actions of Ionizing Radiation on Oligopeptide
Derivatives of Glycine in the Neutral (Zwitterion) and Basic Forms

Warren M. Garrison* and Harvey A. Sokol

Materials and Molecular Research Division
Lawrence Berkeley Laboratory
University of California
Berkeley, CA 94720

ABSTRACT

Effects of protonation of the terminal NH_2 group on the relative

yields of reductive deamination and deamidation by e^- in the

γ-radiolysis of di, tri and tetra glycine in the solid state are

described. The experimental data provide direct chemical evidence of

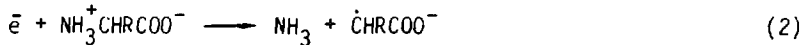specific sites of addition of e^- to $C = O$ bonds along the peptide

chain.

Radiolysis of the simpler aliphatic α-amino acids leads to deamination as a major chemical consequence both in aqueous solution and in the solid state. Detailed reaction mechanisms for the radiolysis of these systems have been developed.[1,2]

In the γ-radiolysis of the neutral (zwitterion) form of glycine and alanine, for example, the ionization step

$$NH_3^+CHRCOO^- \xrightarrow{\hspace{1cm}} NH_3^+\dot{C}RCOO^- + H^+ + e^- \tag{1}$$
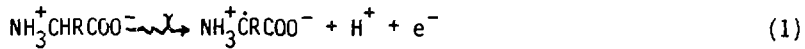
is immediately followed by

$$\bar{e} + NH_3^+CHRCOO^- \longrightarrow NH_3 + \dot{C}HRCOO^- \tag{2}$$

Subsequent radical removal steps include

$$\dot{C}HRCOO^- + NH_3^+CHRCOO^- \longrightarrow CH_2RCOO^- + NH_3^+\dot{C}RCOO^- \tag{3}$$

$$2\ NH_3^+\dot{C}RCOO^- \longrightarrow NH_2^+{=}CRCOO^- + NH_3^+CHRCOO^- \tag{4}$$
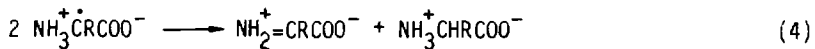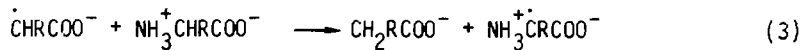
Hydrolysis of the labile iminoacid, $NH_2^+{=}CRCOO^-$, occurs on dissolution of the irradiated solid in water prior to chemical analysis,

$$H_2O + NH_2^+{=}CRCOO^- \longrightarrow NH_4^+ + RCOCOO^- \tag{5}$$
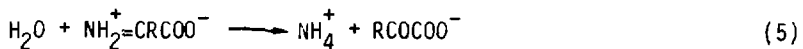
With γ-rays, the chemistry of reactions 1-5 gives the product stoichiometry $G(NH_3) \approx 5$, $G(RCOCOOH) \approx 2.5$, $G(RCH_2COOH) = 2.5$[1,3] where G represents the number of product molecules formed per 100 eV absorbed energy.

The evidence is that the reductive deamination of an amino acid zwitterion via reaction 2 involves addition of e^- to the C – O double bond of the carboxyl group and that dissociation of the N-C bond then ensues[2,3]

$$e^- + NH_3^+CHRCOO^- \longrightarrow NH_3^+CHR\dot{C}\begin{matrix} O^- \\ O^- \end{matrix} \qquad (6)$$

$$NH_3^+CHR\dot{C}\begin{matrix} O^- \\ O^- \end{matrix} \longrightarrow NH_3 + \dot{C}HRCOO^- \qquad (7)$$
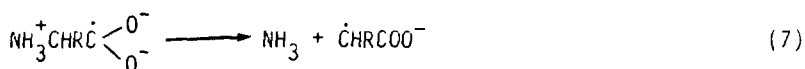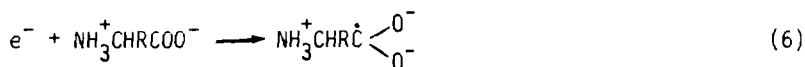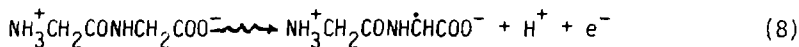
The unsaturated double bond must be present α to the NH_3^+ group for reductive deamination to occur. Simple unsubstituted aliphatic amines (NH_3^+R) and β-amino acids $(NH_3^+CH_2CH_2COO^-)$, for example, do not undergo this reaction.

Subsequent work[4,5] has established that the linear di, tri and tetra peptide derivatives of glycine and alanine undergo reductive deamination analogous to that formulated in equations 6,7. In the γ-radiolysis of solid diglycine (glycylglycine) the observed chemistry is in accord with the reaction sequence

$$NH_3^+CH_2CONHCH_2COO^- \rightsquigarrow NH_3^+CH_2CONH\dot{C}HCOO^- + H^+ + e^- \qquad (8)$$

$$e^- + NH_3^+CH_2CONHCH_2COO^- \longrightarrow NH_3 + \overset{\cdot}{C}H_2CONHCH_2COO^- \qquad (9)$$

$$\overset{\cdot}{C}H_2CONHCH_2COO^- + NH_3^+CH_2CONHCH_2COO^- \longrightarrow CH_3CONHCH_2COO^- + NH_3^+CH_2CONH\overset{\cdot}{C}HCOO^- \quad (10)$$
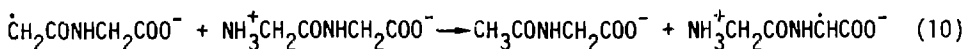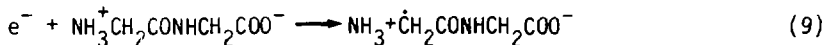
to give $G(NH_3) \simeq G(\text{N-acetylglycine}) \simeq 4.$[4] The long-lived peptide radicals formed in Steps 8, 10 are removed predominantly through dimerization to yield higher molecular weight derivatives of α,α'-diaminosuccinic acid.
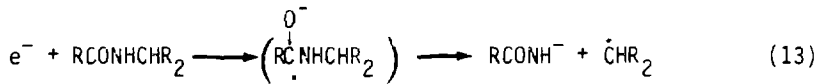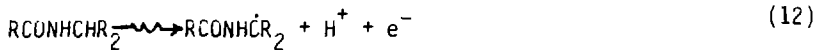
The yields of free ammonia and the corresponding N-acetyl derivative from di, tri and tetra glycine are summarized in Table I. It is seen that cleavage of the terminal N-C bond occurs preferentially in the radiolysis of this homologous series of linear peptides. The yield of free ammonia and the corresponding N-acetyl derivative does, however, decrease gradually with increasing molecular weight of the peptide. With polyglycine and polyalanine (MW ~ 2000) the yield of free ammonia decreases to a value of $G \simeq 0.5$.

This decrease in the yield of free ammonia with increasing chain length indicates (a) that peptide $C = O$ groups in addition to the one α to the NH_3^+ group can compete as trapping centers for e^- and (b) that such trapping does not lead to the formation of free ammonia. Other radiation chemical studies support this interpretation. For example, some years ago it was shown[6] that a major overall reaction in the radiolysis of polyamino acids leads to cleavage of the peptide main chain

$$3RCONHCHR_2 \xrightarrow{\hspace{0.5cm}} R\dot{C}ONH_2 + \dot{C}H_2R_2 + 2RCON\dot{H}CR_2 \qquad \cdot \qquad (11)$$
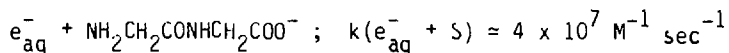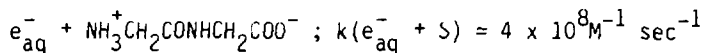
to give amide and N-acyl derivatives as the major degradation
products. More recent ESR studies[7] indicate that the over-all
reaction involves the steps

$$RCONHCHR_2 \xrightarrow{\hspace{0.5cm}} RCONH\dot{C}R_2 + H^+ + e^- \qquad (12)$$

$$e^- + RCONHCHR_2 \longrightarrow \left(R\overset{O^-}{\underset{\cdot}{\dot{C}}} NHCHR_2 \right) \longrightarrow RCONH^- + \dot{C}HR_2 \qquad (13)$$

In other words, trapping of e^- at $C = O$ bonds along the peptide
chain leads to deamidation as formulated in steps 11-13 while trapping
of e^- at the $C = O$ bond α to the terminal NH_3^+ group leads to
deamination via steps 8-10 above.

Recent pulse radiolysis studies[8] have shown that the rates of
reaction of the hydrated electron (e_{aq}^-) with the zwitterion forms
of di, tri and tetra glycine are from 10^1 to 10^2 times faster than
the reaction of e_{aq}^- with the corresponding basic forms. For
example with glycyglycine:

$$e_{aq}^- + NH_3^+CH_2CONHCH_2COO^- \; ; \; k(e_{aq}^- + S) \simeq 4 \times 10^8 M^{-1} \; sec^{-1}$$

$$e_{aq}^- + NH_2CH_2CONHCH_2COO^- \; ; \; k(e_{aq}^- + S) \simeq 4 \times 10^7 \; M^{-1} \; sec^{-1}$$

To obtain information on the effects of protonation of the NH_2 group
in the γ-radiolysis of solid oligopeptides, we have measured the

relative yields of <u>deamination</u> and <u>deamidation</u> with zwitterion and basic (i.e., sodium salt) forms of di, tri and tetraglycine. The data are summarized in Tables IIA, IIB.

From Table IIA it is clear that reductive deamination via reaction 9 predominates with diglycine in both the zwitterion and basic forms. However, as the length of the chain increases, the yield of deamination rapidly <u>decreases</u> with the basic forms as compared to the zwitterion forms. And, as shown in Table IIB this decrease is accompanied by a marked increase in the deamidation reaction of type 11-13. These findings provide direct chemical evidence that the $C = O$ bonds along the peptide chain represent competing chemical traps for e^- and that protonation of the terminal NH_2 group preferentially enhances the reactivity of the $C = O$ bond in the adjacent α position.

EXPERIMENTAL

The di, tri and tetraglycine derivatives were obtained from Nutritional Biochemicals and were re-crystallized from distilled water to remove traces of free ammonia. Sodium salts were prepared by adding the solid peptides to a stoichiometric volume of reagent grade 0.1 N NaOH. These solutions were then evaporated to a constant weight on the vacuum line. The salts were irradiated in vacuo and dissolved in water under nitrogen for subsequent chemical analysis.

Irradiations were made with ^{60}Co γ-ray at dose rates of 5 x 10^{17} eV/g-min. Dosage was determined with the Fricke dosimeter [G(Fe^{3+} = 15.5]. All yields are expressed as G values (molecules per 100 eV absorbed energy). Energy deposition in the solids was taken to be proportional to electron density. Free ammonia was determined by use of a modification of the microdiffusion method of Conway.[5] The N-acetyl products were separated by passing the sample solution through Dowex 50 in the acid form. The N-acetyl products pass through the column with little or no fractionation or retention. The effluent was lyophilized to dryness and the acetyl products in the residue were identified and assayed through application of the techniques of filter-paper chromatography.[9]

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Table I. Product Yields in the γ-Radiolysis of Oligopeptide
Derivatives of Glycine (Neutral Zwitterion)

Compound	Yield G	
	Ammonia	N-acetylderivative
Diglycine	4.5	3.4
Triglycine	3.1	3.2
Tetraglycine	2.3	2.3
Polyglycine	~0.5	---

Table II. Comparative Product Yields in the γ-Radiolysis of Oligo-
peptide Derivatives of Glycine in the Zwitterion and Basic
(sodium salt) Forms.

A. Deamination (NH_2‒CH_2~)

	Product	Zwitterion	Yield G Sodium Salt
Diglycine	N‒acetylglycine	3.4	2.2
Triglycine	N‒acetyldiglycine	3.2	0.5
Tetraglycine	N‒acetyltriglycine	2.3	0.5

B. Deamidation (~$CONH$‒CH_2~)

	Product	Zwitterion	Sodium Salt
Diglycine	acetic acid	<0.1	0.4
Triglycine	N‒acetylglycine	<0.1	2.0
Tetraglycine	N‒acetyldiglycine	<0.1	1.5