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BASIC FORMS

Warren M. Garrison and Harvey A. Sokol

January 1980

MASTER

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

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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 $\text{C}=\text{O}$ bonds along the peptide chain.

DISCLAIMER

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This work was supported by the U. S. Department of Energy under Contract W-7405-ENG-48

DESCRIPTION OF THE ...

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

is immediately followed by

Subsequent radical removal steps include

Hydrolysis of the labile iminoacid, $\text{NH}_2^+=\text{CRCOO}^-$, occurs on dissolution of the irradiated solid in water prior to chemical analysis,

With γ -rays, the chemistry of reactions 1-5 gives the product stoichiometry $G(\text{NH}_3) \approx 5$, $G(\text{RCO}_2\text{COOH}) \approx 2.5$, $G(\text{RCH}_2\text{COOH}) = 2.5^{1,3}$ 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}

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 ($\text{NH}_3^+\text{CH}_2\text{CH}_2\text{COO}^-$), 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

to give $G(\text{NH}_3) \approx G(\text{N-acetylglycine}) \approx 4$.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 $G \approx 0.5$.

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 shown⁶ that a major overall reaction in the radiolysis of polyamino acids leads to cleavage of the peptide main chain

to give amide and N-acyl derivatives as the major degradation products. More recent ESR studies⁷ indicate that the over-all reaction involves the steps

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 studies⁸ 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 glycylglycine:

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 deamination and deamidation 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 decreases 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 5×10^{17} eV/g-min. Dosage was determined with the Fricke dosimeter [$G(\text{Fe}^{3+}) = 15.5$]. 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-acetyl derivative
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 Oligopeptide Derivatives of Glycine in the Zwitterion and Basic (sodium salt) Forms.

		<u>Yield G</u>	
Product		Zwitterion	Sodium Salt
A. Deamination ($\text{NH}_2\text{-CH}_2\text{-}$)			
Diglycine	N-acetylglycine	3.4	2.2
Triglycine	N-acetyldiglycine	3.2	0.5
Tetraglycine	N-acetyltriglycine	2.3	0.5
B. Deamidation ($\text{-CONH-CH}_2\text{-}$)			
Product			
Diglycine	acetic acid	<0.1	0.4
Triglycine	N-acetylglycine	<0.1	2.0
Tetraglycine	N-acetyldiglycine	<0.1	1.5