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DISCLAIMER

REDUCTIVE DEAMINATION IN THE RADIOLYSIS OF OLIGOPEPTIDES IN AQUEOUS SOLUTION AND IN THE SOLID STATE[1,2]

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ABSTRACT

Chemical trapping of e^- at the carboxyl group of the α-amino acids leads to formation of ammonia and fatty acids via dissociative cleavage of the N-C bond: $e^- + NH_3^+CHRCO_2^- \rightarrow (NH_3^+\dot{C}HRCO_2^{=}) \rightarrow NH_3 + \dot{C}HRCO_2^-$. Such reaction represents a major path for removal of e^- formed in the γ-radiolysis of glycine and alanine both in aqueous solution and in the solid state. We have now investigated the rôle of reductive deamination in the γ-radiolysis of the di and tri peptide derivatives of glycine and alanine. The evidence is that in these systems the peptide linkage represents the effective trapping center: $e^- + NH_3^+CHRCONHCHR_2 \rightarrow (NH_3^+\dot{C}HR(O^-)NHCHR_2) \rightarrow NH_3 + \dot{C}HRCONHCHR_2$. In the γ-radiolysis of dilute, O_2-free solutions of glycylglycine and alanylalanine we obtain $G(NH_3)_{free} \simeq G(\text{acylamino acid}) \simeq 3 \simeq G_{e^-}$. The same products in essentially the same yield are also obtained in the γ-radiolysis of these dipeptides in the solid state. The tripeptide derivatives show analogous reactions. A detailed comparative analysis of the radiation chemistry of the amino acids, glycine and alanine, and their respective peptide derivatives is given.

Radiolysis of the simpler α-amino acids leads to deamination as a major chemical consequence both in aqueous solution and in the solid state.[3]

In aqueous solutions of glycine and alanine the ionization step[4]

$$H_2O \rightsquigarrow H^+, OH, e^-_{aq} \quad ,$$

is followed by[3d,5]

$$e^-_{aq} + NH_3^+CHRCOO^- \longrightarrow NH_3 + \dot{C}HRCOO^- \qquad (1)$$

$$OH + NH_3^+CHRCOO^- \longrightarrow H_2O + NH_3^+\dot{C}RCOO^- \quad . \qquad (2)$$

Subsequent steps yield fatty acid and keto acid as major organic products[3d]

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

$$2\ NH_3^+\dot{C}RCOO^- \longrightarrow NH_2^+{=}CRCOO^- + NH_3^+CHRCOO^- \qquad (4)$$

$$H_2O + NH_2^+{=}CRCOO^- \longrightarrow NH_4^+ + RCOCOO^- \quad , \qquad (5)$$

to give $G(NH_3) \simeq G(\text{fatty acid}) + G(\text{keto acid}) \simeq 5$.

The yield for reductive deamination by e^-_{aq} can be measured directly through use[3d] of second solutes which are highly reactive toward OH but relatively unreactive toward e^-_{aq}. Formate ion[6] is such a solute and the effects of increasing formate concentration on product yields from 0.5 M glycine solution are shown in Fig. 1. We see that $G(NH_3)$ drops rapidly with increasing formate concentration and then levels off at a limiting value which is a measure of the reductive deamination reaction 1. Consistent with this is the finding that the keto acid yield goes to zero with increasing formate while the fatty acid yield is essentially unaffected as shown. Similar results are obtained with other simple aliphatic α-amino acids.

Reductive deamination is not, however, a general and characteristic reaction of amines per se. Simple unsubstituted aliphatic amines[7] and β-amino acids,[3d,8] for example, do not show the reaction. The evidence is that for reductive deamination by e^-_{aq} to occur, an unsaturated double bond must be present α to the NH_3^+ group.[3d,8] The electron adds to the double bond and dissociation of the N-C linkage ensues. For the α-amino acids we write[5,9]

$$e^-_{aq} + NH_3^+CHRC(=O)O^- \longrightarrow NH_3^+CHRC(\cdot)(O^-)O^- \qquad (6)$$

$$NH_3^+CHRC(\cdot)(O^-)O^- \longrightarrow NH_3 + \dot{C}HRC(=O)O^- \qquad (7)$$

These observations suggested to us that reactions analogous to steps 6, 7 are also involved in the radiolysis of these compounds in the solid state.[5] And, recent chemical[10,11] and physical[12,13] observations indicate such reactions are indeed of importance in the γ-radiolysis of the simple α-amino acids as solids. The observed chemistry conforms to the over-all reaction scheme

$$NH_3^+CHRCOO^- \rightsquigarrow NH_3^+\dot{C}RCOO^- + H^+ + e^- \qquad (8)$$

$$e^- + NH_3^+CHRCOO^- \longrightarrow NH_3 + \dot{C}HRCOO^- \qquad (9)$$

$$\dot{C}HRCOO^- + NH_3^+CHRCOO^- \longrightarrow CH_2RCOO^- + NH_3^+\dot{C}RCOO^- \qquad (10)$$

$$2\ NH_3^+\dot{C}RCOO^- \longrightarrow NH_2^+{=}CRCOO^- + NH_3^+CHRCOO^- \qquad (11)$$

$$H_2O + NH_2^+{=}CRCOO^- \longrightarrow NH_4^+ + RCOCOO^- \quad . \qquad (12)$$

We now find that the linear di, tri, and tetra peptide derivatives of glycine and alanine undergo analogous reductive deamination reactions.

In the γ-radiolysis of these oligopeptides in oxygen-free solution we find[14] $G(NH_3) \simeq 3$ at solute concentrations above 0.05 M. Concentration yield curves for diglycine and triglycine are shown in Fig. 2. Addition of formate to these systems results in a small decrease in $G(NH_3)$ but the effect is not large even at formate concentrations sufficient to quantitatively scavenge the OH radicals as shown in Fig. 3. The evidence is (a) that essentially all of the free ammonia liberated in radiolysis of these oligopeptides arises as a consequence of reductive deamination and (b) that e^-_{aq} in all of these systems is preferentially trapped at the C=O function of the peptide linkage α to the NH_3^+ group. Radiolysis of these oligopeptides in aqueous solution may be formulated as follows

$$H_2O \xrightarrow{\sim\sim} H^+,\ OH,\ e^-_{aq}$$

$$e^-_{aq} + NH_3^+CHRCONHCHR_2 \longrightarrow NH_3 + \dot{C}HRCONHCHR_2 \qquad (13)$$

$$OH + NH_3^+CHRCONHCHR_2 \longrightarrow NH_3^+CHRCONH\dot{C}R_2 + H_2O \qquad (14)$$

$$\dot{C}HRCONHCHR_2 + NH_3^+CHRCONHCHR_2 \longrightarrow CH_2RCONHCHR_2 + NH_3^+CHRCONH\dot{C}R_2 \qquad (15)$$

where the peptide radicals $NH_3^+CHRCONH\dot{C}R_2$ formed in reactions 14 and 15 are subsequently removed through dimerization (cross-linking) to yield the diaminosuccinic acid derivative.[15] In accordance with the above formulation we find that acetylglycine and acetylglycylglycine are formed as major products in the radiolysis of aqueous diglycine and triglycine respectively.[16] Hydrolysis[17] of the irradiated solutions liberates acetic acid in the yields shown in Table I.

Corresponding data for the solid state systems are shown in Table II. Here again acetylglycine and acetylglycylglycine are formed as major products from diglycine and triglycine. The products of Table II may be accounted for in terms of the formulation,

$$NH_3^+CHRCONHCHR_2 \rightsquigarrow NH_3^+CHRCONH\dot{C}R_2 + H^+ + e^- \quad (16)$$

$$e^- + NH_3^+CHRCONHCHR_2 \longrightarrow NH_3 + \dot{C}HRCONHCHR_2 \quad (17)$$

$$\dot{C}HRCONHCHR_2 + NH_3^+CHRCONHCHR_2 \longrightarrow CH_2RCONHCHR_2 + NH_3^+CHRCONH\dot{C}R_2 \quad (18)$$

where $NH_3^+CHRCONH\dot{C}R_2$ represents the long-lived peptide radical observed at room temperature by esr methods.[18]

Although both diglycine and triglycine on irradiation as solids give $G(NH_3) \simeq 3$, the value decreases to $G(NH_3) \simeq 2.3$ with tetraglycine. With polyalanine (MW-2000), $G(NH_3) \simeq 0.5$. With increasing molecular weight C=O groups at peptide linkages other than the one α to the terminal NH_3^+ group compete as trapping centers for e^-. Such trapping along the peptide chain does not however lead to chain cleavage as has been noted elsewhere.[19]

FOOTNOTES AND REFERENCES

1. Work performed under the auspices of the U. S. Atomic Energy Commission.

2. Prepared for presentation at the 18th Annual Meeting of the Radiation Research Society, Dallas, March 1-5, 1970.

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6. $k(HCOO^- + OH) \simeq 2.5 \times 10^9\ M^{-1}sec^{-1}$, $k(HCOO^- + e^-_{aq}) = < 10^6\ M^{-1}sec^{-1}$; see the review by M. Anbar and P. Neta, Intern. J. Appl. Radiation Isotopes 18, 493 (1967).

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14. Determined after the method of E. J. Conway and A. Byrne, Biochem. J. 27, 419 (1933).

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16. The irradiated solutions were passed through Dowex 50 (acid form) to remove the oligopeptide. The effluent containing the acetyl derivative was evaporated to dryness. The product derivative was transferred to filter paper in methanol and chromatographed with the butanol-ammonia solvent system (Ref. 15) in parallel with authentic material. The irradiated solid systems received the identical treatment after dissolution in water under a nitrogen atmosphere.

17. Under nitrogen in 1 N H_2SO_4 at 95°C for 17 hours.

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Table I. Product yields in the γ-radiolysis of diglycine and triglycine in 0.1 M oxygen-free solutions.

Compound	Yield, G	
	Ammonia	Acetyl derivative
Diglycine	3.1	2.5
Triglycine	2.8	2.0

Table II. Product yields in the γ-radiolysis of diglycine and triglycine in the solid state (evacuated).

Compound	Yield, G	
	Ammonia	Acetyl derivative
Diglycine	4.5	3.4
Triglycine	3.1	3.2

FIGURE CAPTIONS

Fig. 1. Product yields from 1.0 M alanine as a function of sodium formate concentration in oxygen-free solution of pH 6.4 under γ radiolysis. Ammonia (●), propionic acid (▲), and pyruvic acid (■) (Ref. 3d).

Fig. 2. Effect of diglycine and triglycine concentrations on ammonia yields from oxygen-free solutions at pH 5.8 under γ radiolysis.

Fig. 3. Effect of formate concentration on ammonia yields in the γ radiolysis of 1.0 M glycine (○) and 0.20 M glycylglycine (●) in oxygen-free solution at pH 6.5 (Ref. 8).

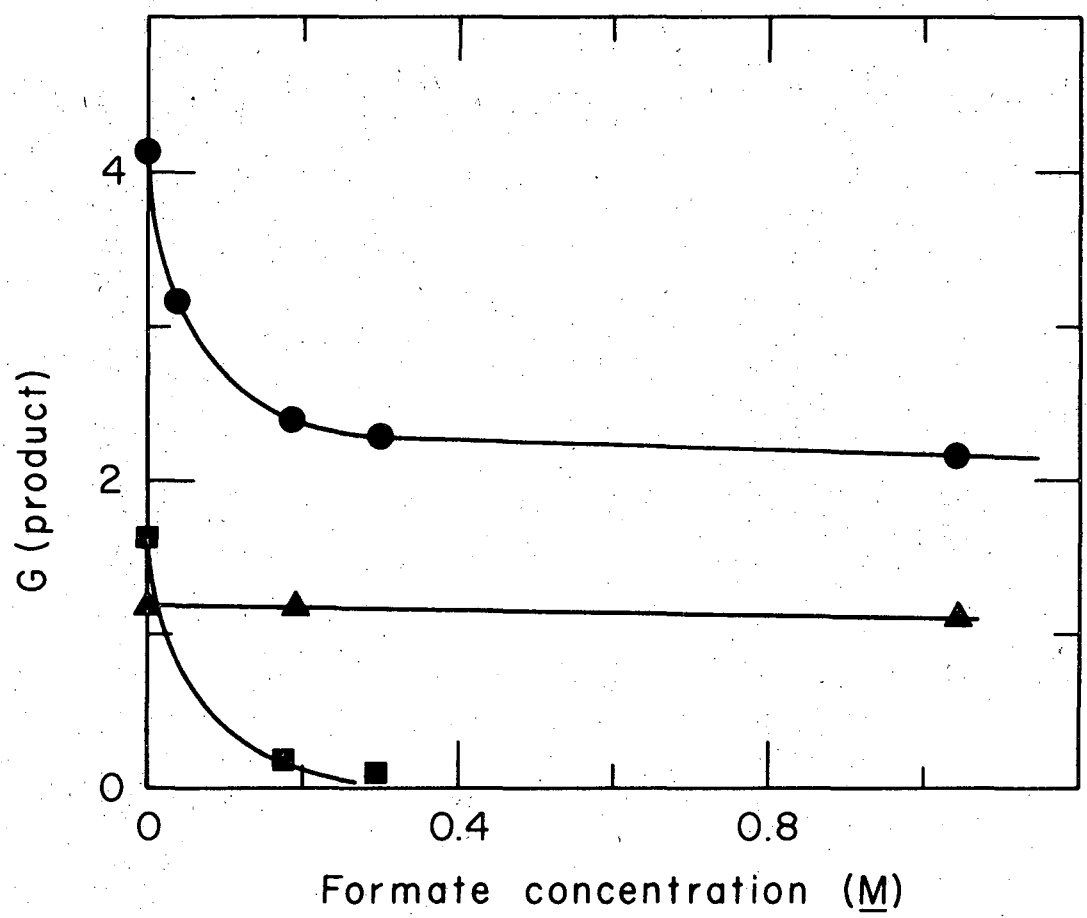

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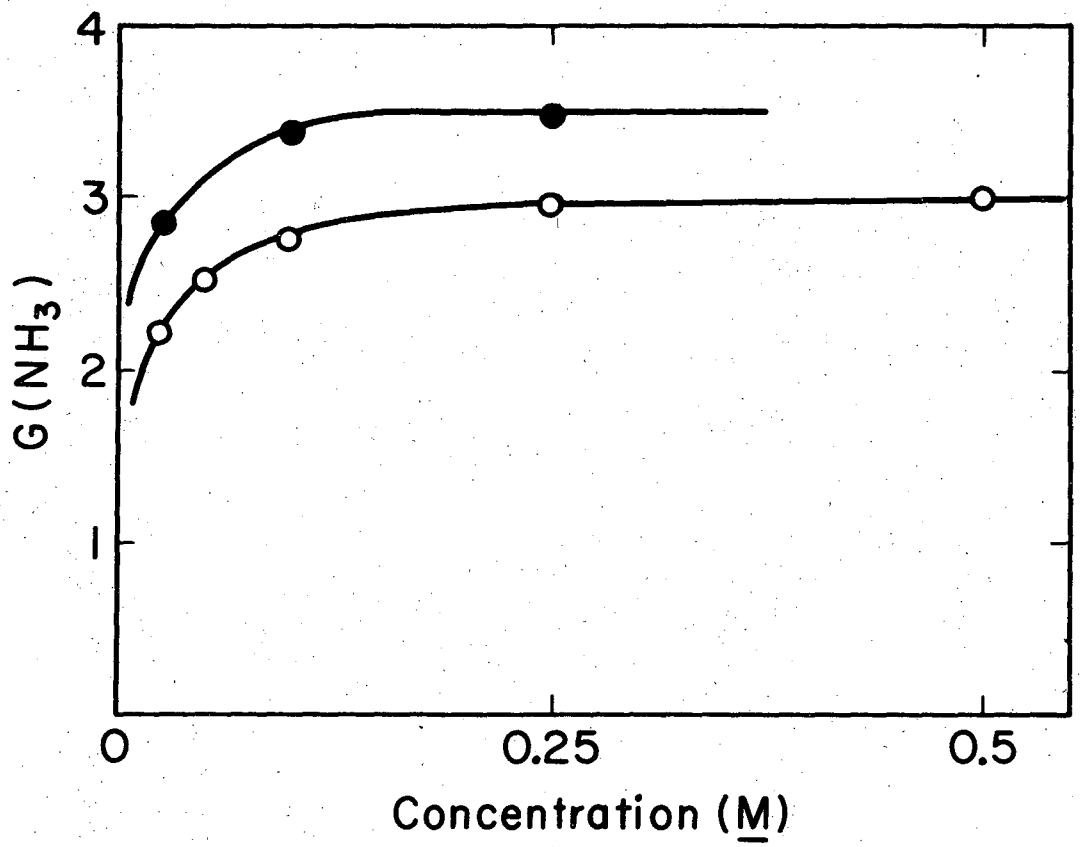
Fig. 1



XBL702-2351

Fig. 2

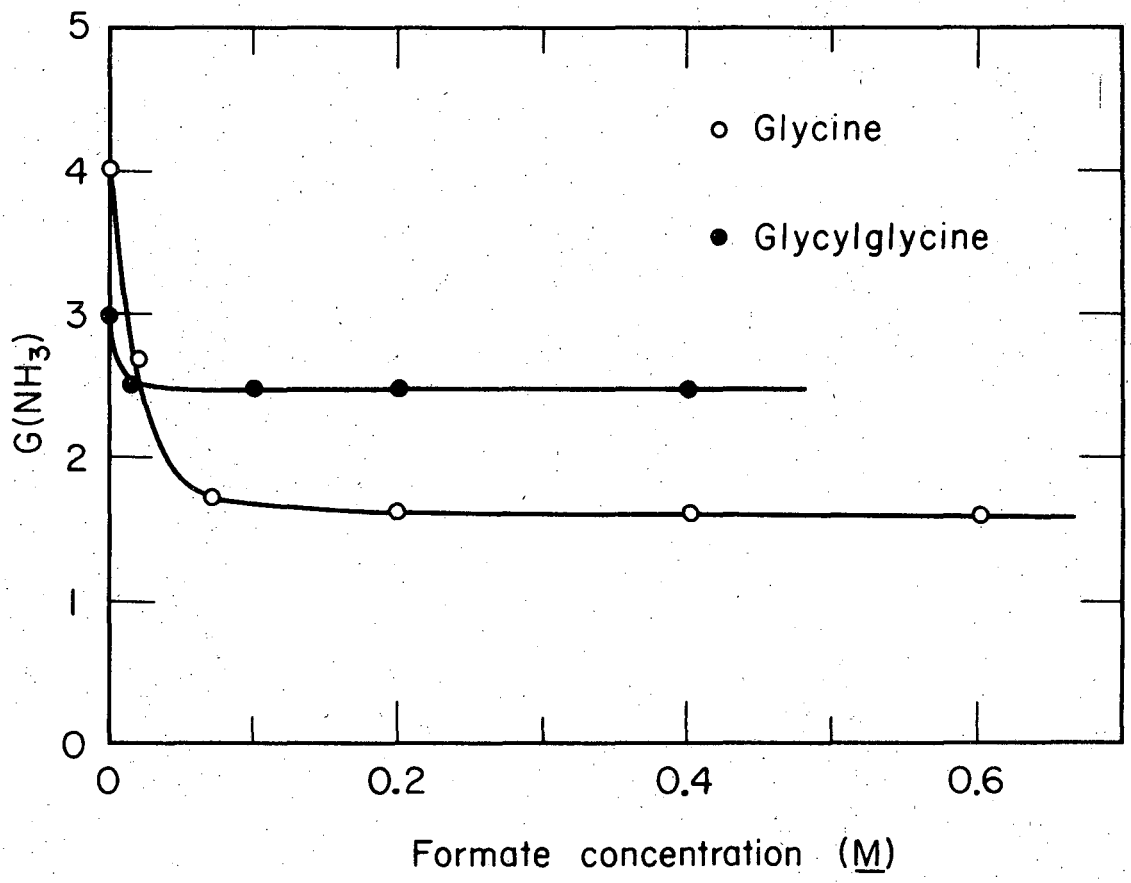

5
4
3
2
1
0
G(NH3)
0
0.2
0.4
0.6
Formate concentration (M)
Glycine
Glycylglycine
MUB-13911

Fig. 3

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