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THE RADIATION-INDUCED "HYDROLYSIS" OF THE PEPTIDE BOND[1]

Michael A. J. Rodgers, Harvey A. Sokol, and Warren M. Garrison

Lawrence Radiation Laboratory
University of California
Berkeley, California

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We find that the γ-radiolysis of oxygen-free solutions of simple peptides such as the N-acetylamino acids leads to liberation of the free amino acid as a major reaction product. As a specific example, the alanine[2] yield from N-acetylalanine in evacuated solution at pH 7 increases abruptly with increasing solute concentration over the range .05M to 0.25M and then levels off to a limiting value of G(alanine) ≃ 1 at acetylalanine concentrations above 0.5M.

This radiation-induced liberation of free amino acid is quenched by second solutes such as hydronium ion, molecular oxygen, and chloracetate ion all of which are known to be effective scavengers of the hydrated electron, $\bar{e}_{aq}$, formed in the radiation-induced step[3,4]

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

The effect of chloracetate ion on G(alanine) from 1M acetylalanine at pH 7 is shown in Fig. 1. The reciprocal-yield plot (Fig. 1, insert) gives $k_2/k_3 = 1.9 \times 10^2$ for the ratio of the rate constants of the competing reactions

$$e^-_{aq} + RCl \rightarrow R + Cl^- \qquad (2)$$

$$e^-_{aq} + RCONHCHR_2 \rightarrow (RCONHCHR_2)^- \rightarrow \text{products} \qquad (3)$$

This value is in good agreement with other measurements of the reactivity of e^-_{aq} towards these solutes.[5]

The evidence is then that the reducing species, e^-_{aq}, is specifically involved in the chemistry that leads to liberation of free alanine. Previous work has established that the oxidizing species, OH, is removed preferentially through H-abstraction at the α-carbon position of acetylalanine

$$OH + RCONHCHR_2 \rightarrow RCONH\dot{C}R_2 + H_2O \qquad (4)$$

where $k_4 = 2.5 \times 10^8\ M^{-1}\ sec^{-1}$.[6] Addition of an OH scavenger such as formate ion ($k_{f+OH} = 2.5 \times 10^9\ M^{-1}\ sec^{-1}$)[4] at concentrations as high as 0.5 M has no effect on G(alanine) in 1M acetylalanine.

Now, if the removal of e^-_{aq} via reaction 3 leads to reduction of the peptide linkage e.g.

$$(RCONHCHR_2)^- + H_2O \rightarrow R\dot{C}(OH)NHCHR_2 \qquad (5)$$

then it is clear that combination of $R\dot{C}(OH)NHCHR_2$ with like species or with the α-carbon radical $RCONH\dot{C}R_2$ would lead to formation of Schiff-base derivatives of the type $R(R')C(OH)NHCHR_2$ which compounds are labile with respect to the decomposition

$$R(R')C(OH)NHCHR_2 \rightarrow R(R')CO + NH_2CHR_2 \qquad (6)$$

The stoichiometry of reaction 6 would also be observed if the (Schiff-base) radical $R\dot{C}(OH)NHCHR_2$ underwent the decomposition

$$R\dot{C}(OH)NHCHR_2 \rightarrow R\dot{C}O + NH_2CHR_2 \qquad (7)$$

before dimerization. In any event, the main point here is that the formation of alanine through net reduction of the peptide linkage in accord with the stoichiometry of reactions 6 and 7 requires the concomitant formation of ketonic products R(R')CO. Detailed chemical analyses[7] of the irradiated solutions reveal that the yield of such products is quite low with $G \leq 0.2$. Evidently reductive cleavage of the peptide bond does not yield the major part of the observed alanine even though the formation of this product is directly related to the attack of the reducing species, e^-_{aq}.

A concept that appears to provide a clue to the interpretation of the chemistry of present system, is that the radical products of e^-_{aq} and OH attack are subsequently removed not by dimerization (combination) but by disproportionation. Of course, disproportionation involving $R\dot{C}(OH)NHCHR_2$ and the α-carbon radical would lead simply to a reconstitution of the parent peptide,

$$R\dot{C}(OH)NHCHR_2 + RCONH\dot{C}R_2 \rightarrow 2\ RCONHCHR_2 \qquad (8)$$

However since the reduced radical $R\dot{C}(OH)NHCHR_2$ is an amine species it is likely to be in an adduct form in the presence of high concentrations

of the peptide

$$\dot{RC}(OH)NHCHR_2 + RCONHCHR_2 \rightleftarrows \underset{\underset{RC(OH)NHCHR_2}{|}}{\dot{RC}(OH)NCHR_2} \qquad (9)$$

(I)

Removal of the adduct radical (I) via the analogue of reaction 8 leads to formation of a Schiff-base (I) which as shown in Eq. 10 can rearrange to give alanine and diacetylalanine[8]

$$\underset{\underset{RC(OH)NHCHR_2}{|}}{RCONCHR_2} \longrightarrow (R_2CO)_2NCHR_2 + NH_2CHR_2 \qquad (10)$$

(I)

The diacetylamines are labile with respect to the hydrolysis

$$(R_2CO)_2NHCHR_2 + H_2O \longrightarrow RCOOH + RCONHCHR_2 \qquad (11)$$

Our measurements of G(acetic) are in good agreement with this formulation. We find that the yield of free acetic acid is low, $G \leq 0.3$, and, we also find that on mild differential hydrolysis of the irradiated solution additional acid is liberated to give G(acetic) $\simeq 1.5 \simeq$ G(alanine).

A detailed account of this work is in preparation and will appear in a forthcoming publication.

Footnotes and References

(1) This work was done under the auspices of the U. S. Atomic Energy Commission.

(2) Alanine was identified chromatographically and assayed by the ninhydrin method; since ammonia is produced as a minor product and is "ninhydrin-positive," a correction was made on the basis of a standard ammonia assay after the method of Conway.

(3) C. J. Hochanadel and R. Casey [Radiation Res. 25 198 (1965)] report the following 100 ev yield for reaction 1: $G_{OH}=2.59$, $G_{e^-_{aq}}=2.58$, $G_H=0.55$, $G_{H_2}=0.45$, $G_{H_2O_2}=0.72$.

(4) For a recent compilation of rate data see: M. Anbar and P. Neta, J. Appl. Radiat. Isotopes 17 493 (1967).

(5) R. L. S. Willix and W. M. Garrison, UCRL-17285, Radiation Res., in press.

(6) M. A. J. Rodgers and W. M. Garrison, UCRL-17886, J. Phys. Chem., submitted.

(7) a. B. M. Weeks, S. A. Cole, and W. M. Garrison, J. Phys. Chem. 69 4131 (1965),
b. W. M. Garrison and B. M. Weeks, Radiation Res., Suppl. 4, 54 (1964).

(8) Product I could, of course, be formed through combination of $R\dot{C}(OH)NHCHR_2$ with nitrogen radicals of the type RCONCHR. The latter are not produced through OH attack (ref. 7b) but we cannot rule out the possibility that such species may be formed through "direct-action" processes at high solute concentrations.

Figure Captions

Fig. 1. G(alanine) as a function of acetylalanine concentration in oxygen-free solution at pH 7 under γ rays. Insert: Reciprocal yield plot of G(alanine) as a function of chloracetate concentration in 1M acetylalanine.

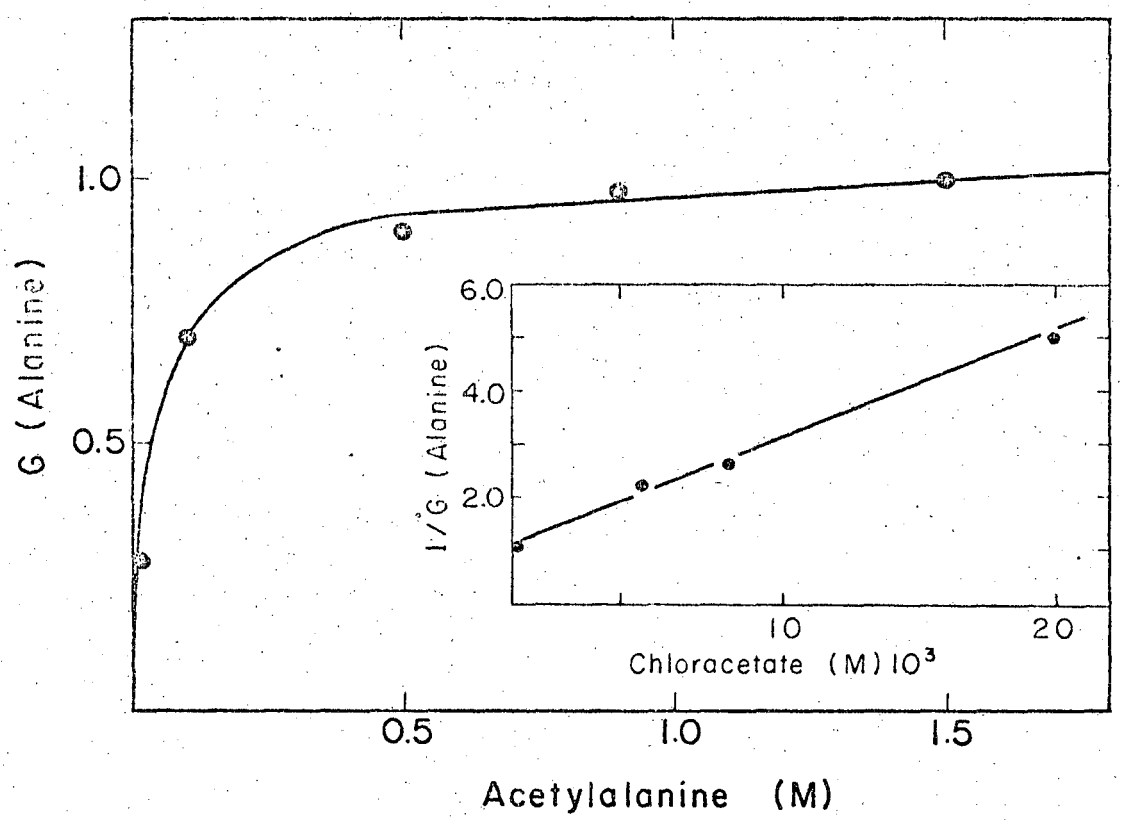

Figure 1

G(alanine) as a function of acetylalanine concentration in oxygen-free solution at pH 7 under γ rays. Insert: Reciprocal yield plot of G(alanine) as a function of chloracetate concentration in 1M acetylalanine.

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