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Authors
Kland-English, M.J.
Sokol, H.A.
Garrison, W. M.

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LAWRENCE RADIATION LABORATORY
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RADIOLYTIC OXIDATION OF THE PEPTIDE MAIN-CHAIN IN DILUTE AQUEOUS SOLUTION: CHEMISTRY OF THE PEROXY RADICAL, $RCONHC(\dot{O}_2)R_2$[1,2]

M. J. Kland-English, H. A. Sokol, and W. M. Garrison

Lawrence Radiation Laboratory
University of California
Berkeley, California 94720

July 1969

ABSTRACT

In the γ-radiolysis of peptides in dilute O_2-saturated solution, reaction of OH radicals at the main-chain leads to formation of the long-lived peroxy radicals $RCONHC(\dot{O}_2)R_2$. Subsequent reactions of these radicals lead to main-chain cleavage. A number of competing modes of degradation are involved. A proposed scheme of reactions accounts for the observed product stoichiometries.

The radiolytic oxidation of the peptide main-chain in dilute aqueous solution containing O_2 has been described[3] in terms of the radiation-induced step[4,5]

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

followed by

$$OH + RCONHCHR_2 \longrightarrow RCONH\dot{C}R_2 + H_2O \qquad (2)$$

$$O_2 + RCONH\dot{C}R_2 \longrightarrow RCONHC(\dot{O}_2)R_2 \qquad (3)$$

The reducing species formed in reaction (1) are scavenged by O_2 via

$$e^-_{aq}(H) + O_2 \longrightarrow O_2^-(HO_2) \qquad (4)$$

where the products of reaction (4) are related by the equilibrium,[6] $HO_2 \rightleftharpoons H^+ + O_2^-$. The simplest scheme for subsequent radical removal involves

$$HO_2 + RCONHC(\dot{O}_2)R_2 \longrightarrow RCONHC(O_2H)R_2 + O_2 \qquad (5)$$

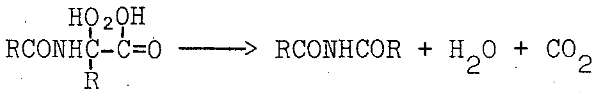
$$H_2O + RCONHC(O_2H)R_2 \longrightarrow RCONH_2 + R_2CO + H_2O_2 \qquad (6)$$

to give amide and carbonyl functions. The yield of reaction (2) is readily followed in terms of free ammonia released on hydrolysis of the irradiated solutions.[3,7,8]

The removal of OH radicals via reaction (2) is essentially quantitative in the γ-radiolysis of various peptide derivatives of the simpler α-amino acids such as glycine and alanine. A plot of $G(NH_3)$ as a function of N-acetylalanine concentration in O_2-saturated solutions levels off at $G(NH_3) \simeq 2.9 \simeq G_{OH}$ in the concentration range 0.05 M to 0.1 M as shown in Fig. 1. At N-acetylalanine concentration above 0.1 M other reaction modes begin to contribute to the observed $G(NH_3)$ values; the chemistry of these other degradation modes in the more concentrated solutions are of quite a different nature as has been described elsewhere.[9]

Now, if degradation of the peptide chain in dilute, oxygenated solutions occurs exclusively through steps (5),(6) we would expect the ammonia and carbonyl yields to be in the relationship $G(NH_3) \simeq G(R_2CO)$. Experimentally we find however, that the ratio $G(R_2CO)/G(NH_3)$ is always less than unity in these systems.[3] With 0.05 M N-acetylalanine, the carbonyl yield[10,11] is extremely low with $G(R_2CO) \leqslant 0.2$.

Further study of the oxidation products formed in the γ-radiolysis of N-acetylalanine solutions reveals that the principal nitrogen-free organic compound produced in this system is acetic acid. Yield data are summarized in Table 1. The finding that acetic acid is produced as a major *initial* product in this system suggested to us that removal of $RCONHC(O_2H)R_2$ via the hydrolytic reaction (6) occurs in competition with a second degradation mode which in the case of N-acetylalanine may be formulated in terms of an intramolecular oxidation, i.e.,



$$\mathrm{RCONH}\overset{\mathrm{HO_2}}{\underset{\mathrm{R}}{\mathrm{C}}}-\overset{\mathrm{OH}}{\mathrm{C}}=\mathrm{O} \longrightarrow \mathrm{RCONHCOR} + H_2O + CO_2 \qquad (7)$$

The diacetamide configuration RCONHCOR is hydrolytically labile and on mild differential hydrolysis[12] of the solution yields acetamide and acetic acid

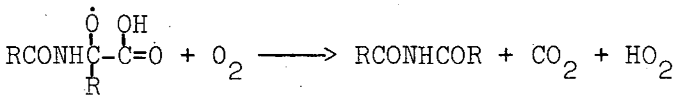
$$H_2O + \mathrm{RCONHCOR} \longrightarrow \mathrm{RCONH_2} + \mathrm{RCOOH} \qquad (8)$$

There is however a difficulty in this interpretation in that it does not account for the fact that both hydrogen peroxide and acetic acid are formed as major products in the radiolysis of dilute oxygenated solutions of N-acetylalanine. The over-all stoichiometry of reactions (1) to (5) followed by reaction (7) requires that $G(H_2O_2) \simeq G_{H_2O_2} \simeq 0.8$. Experimentally[13] however we find that $G(H_2O_2) \simeq 2.2$ as shown in Table 1. Hence we conclude that radical removal via step (5) and the subsequent degradation of $RCONHC(O_2H)R_2$ as formulated in reactions (6),(7) are not predominant reaction modes in the radiolysis of N-acetylalanine under the experimental conditions stipulated in Table 1.

The present evidence is that the $RCONHC(\dot{O}_2)R_2$ radicals derived from N-acetylalanine are removed predominantly via[14]

$$2RCONHC(\dot{O}_2)R_2 \longrightarrow 2RCONHC(\dot{O})R_2 + O_2 \qquad (9)$$

Reaction (9) is then followed by



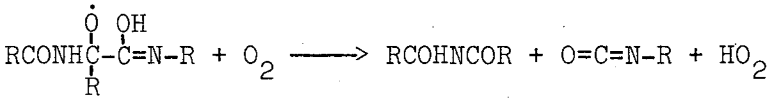
$$RCONH\overset{\dot{O}}{\underset{R}{C}}-\overset{OH}{C}=O + O_2 \longrightarrow RCONHCOR + CO_2 + HO_2 \qquad (10)$$

to give the labile diacetamide derivative. The HO_2 radicals formed via reaction (4) and (10) are removed via

$$2HO_2 \longrightarrow H_2O_2 + O_2 \qquad (11)$$

The sequence of reactions (1) to (4) followed by reactions (9) to (11) satisfies both the qualitative and quantitative requirements of the present system. The data of Table 1 were obtained at pH 3 under which condition the hydroperoxy radical is predominantly in the HO_2 form.[6] Although the yield for main-chain degradation is essentially the same at pH 3 and at pH 7, (Fig. 1) the relative contributions of reactions (6),(7) and (9),(10) may be pH dependent. Such effects are now being evaluated.

For O_2-saturated solutions of poly-DL-alanine we find $G(NH_3) \simeq 3.8$.[15] With this oligopeptide (M.W. 2000) we assume random attack of OH at the main chain and envisage the analogue of reaction (10) as involving the enol form of an adjacent peptide linkage



$$\mathrm{RCONH}\underset{\mathrm{R}}{\overset{\dot{\mathrm{O}}}{\mathrm{C}}}{-}\overset{\mathrm{OH}}{\mathrm{C}}{=}\mathrm{N{-}R} + \mathrm{O_2} \longrightarrow \mathrm{RCOHNCOR} + \mathrm{O{=}C{=}N{-}R} + \mathrm{HO_2} \qquad (12)$$

where

$$\mathrm{O{=}C{=}N{-}R} + \mathrm{H_2O} \longrightarrow \mathrm{CO_2} + \mathrm{RNH_2}$$

follows essentially instantaneously.

Addition of small amounts of Fe(II) to these oxygenated peptide solutions suppresses the production of acetic acid and leads to the formation of ammonia and carbonyl in equal yield. At sufficiently low concentrations Fe(II) does not interfere with reactions (1)-(4) but does intercept the peroxy radicals via[16]

$$\mathrm{RCONHC(\dot{O}_2)R_2} + \mathrm{3Fe(II)} + \mathrm{2H_2O} \longrightarrow \mathrm{RCONHC(OH)R_2} + \mathrm{3Fe(III)} + \mathrm{3OH^-} \qquad (13)$$

$$\mathrm{RCONHC(OH)R_2} \longrightarrow \mathrm{RCONH_2} + \mathrm{R_2CO} \qquad (14)$$

$$\mathrm{HO_2} + \mathrm{Fe(II)} + \mathrm{H_2O} \longrightarrow \mathrm{H_2O_2} + \mathrm{Fe(III)} + \mathrm{OH^-} \qquad (15)$$

Hydrogen peroxide formed in Step (15) is removed via the Fenton reaction[17]

$$\mathrm{H_2O_2} + \mathrm{Fe(II)} \longrightarrow \mathrm{OH} + \mathrm{OH^-} + \mathrm{Fe(III)} \qquad (16)$$

For O_2-saturated, 0.05 M N-acetylalanine we observe the optimum yield at 10^{-3} M Fe(II); i.e., under this condition $G(NH_3) \simeq G(R_2CO) \simeq 7.5$. The carbonyl yield ($R_2CO$) is made up of G(pyruvic) ≃ 5.7, G(acetaldehyde) ≃ 2.0. These findings are in quantitative agreement with the yields of the radiation-induced reaction (1) and the reaction sequence formulated here.[16,18] As would be anticipated on the basis of competition kinetics[19] the oxidation yield goes through a pronounced maximum as the Fe(II) concentration is increased from zero to 10^{-2} M as shown in Fig. 2.

There is no evidence from these radiation-chemical studies that the oxidizing species formed in reaction (16) is other than the free OH radical.[21] In a separate control study of peptide oxidation by Fenton reagent, a dilute solution of H_2O_2 (2×10^{-4} M) was added drop-wise with O_2-stirring to 0.05 M N-acetylalanine solution containing 10^{-3} M Fe(II). We obtained the molecular stoichiometry $-[H_2O_2] = [RCONH_2] = [R_2CO]$ which is in accord with the requirements of the radiolytic studies described above. The ratio G(acetaldehyde)/G(pyruvic) here also is ∿0.30.

FOOTNOTES AND REFERENCES

1. This work was performed under the auspices of the U. S. Atomic Energy Commission.

2. Prepared for presentation at the 158th National Meeting of the American Chemical Society, New York, September 7-12, 1969.

3. For a recent review see W. M. Garrison, Current Topics in Radiation Research, ed. by M. Ebert and A. Howard, Vol. IV, (North-Holland Publishing Co., Amsterdam, 1968) p. 45-94.

4. C. J. Hochanadel and R. Casey, Radiation Res. 25, 198 (1965) report $G_{OH} = 2.59$, $G_{e_{aq}^-} = 2.58$, $G_H = 0.55$, $G_{H_2} = 0.45$, $G_{H_2O_2} = 0.72$. The maximal OH yield at high solute concentration appears to be $G_{OH} \approx 2.9$ (Ref. 5).

5. (a) E. Hayon, Trans. Faraday Soc. 61, 723 (1965), (b) G. Czapski, Adv. Chem Ser. 81, 106 (1968).

6. G. Czapski and H. J. Bielski, J. Phys. Chem. 67, 2180 (1963).

7. Hydrolysis was done in 2N sodium hydroxide at room temperature in the outer compartment of a Conway diffusion cell. Amide hydrolysis and ammonia transfer to the inner chamber (0.1 N sulfuric acid) is complete in 24 hours (Ref. 8).

8. H. L. Atkins, W. Bennett-Corniea, and W. M. Garrison, J. Phys. Chem. 71, 772 (1967). We refer to radical attack at the C-H linkage α to the nitrogen function as the characteristic peptide reaction. With the more complicated amino acid residues OH attack may occur in parallel or preferentially at side-chain loci. See Ref. 3.

9. (a) M. A. J. Rodgers and W. M. Garrison, J. Phys. Chem. 72, 758 (1968).
   (b) W. M. Garrison et al., Adv. Chem. Ser. 81, 384 (1968).

10. Solutions were made 2 N in hydrochloric acid, heated under nitrogen for two min at 95°C, cooled and assayed through use of 2,4-dinitrophenyl hydrazine reagent (Ref. 11).

11. After the method of H. A. Sokol, to be published.

12. The maximal acetic acid yield as measured by v.p. chromatography was obtained after the solutions were made 1 N in sodium hydroxide and allowed to stand at room temperature for 15 minutes. Amide ($RCONH_2$) hydrolysis is negligible under these conditions.

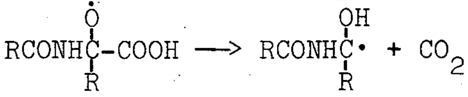
13. Organic peroxides and hydrogen peroxide were determined after the method of G. R. A. Johnson and J. Weiss, Chem. and Ind. p. 358 (1955).

14. In an earlier study we found reaction of the type $2RO_2 \longrightarrow 2RO + O_2$ to be of major importance in the radiolytic oxidation of acetic acid in dilute aqueous solution. [Radiation Res. 10, 273 (1959)]. Such reaction of RO_2 was invoked in earlier studies of radical-initiated oxidation of gaseous hydrocarbons by Vaughan and co-workers [J. Am. Chem. Soc. 73, 15 (1951)].

15. We find (W. Bennett-Corniea, H. A. Sokol, and W. M. Garrison; to be published) that the $G(NH_3)$ values obtained in the γ-radiolysis of dilute oxygenated solutions of oligopeptides and polypeptides are consistently greater than G_{OH}. Our interpretation of this effect is that with such compounds a fraction of the RO radicals formed via reaction (9) react intramolecularly with an adjacent C-H locus of the main chain $RO + RH \longrightarrow ROH + R$. This leads then to an enhancement in the observed yield for amide production. With small molecules containing a single amino acid residue (e.g. N-acetylalanine) the equivalent RO chemistry can only occur intermolecularly and is of negligible importance in dilute solution.

16. Reaction (13) represents a composite reaction which includes the steps: (a) $RO_2 + Fe(II) + H_2O \longrightarrow ROOH + Fe(III) + OH^-$, (b) $ROOH + Fe(II) \longrightarrow RO + OH^- + Fe(III)$, (c) $RO + Fe(II) + H_2O \longrightarrow ROH + Fe(III) + OH^-$.

17. F. Haber and J. Weiss, Proc. Roy. Soc. (London) A147, 333 (1934).

18. The production of acetaldehyde in the N-acetylalanine system we envisage as arising through rearrangement of a fraction of the RO radicals prior to reduction by Fe(II), i.e., specifically (a)  $\mathrm{RCONH}\overset{\dot{O}}{\underset{R}{C}}\mathrm{-COOH} \longrightarrow \mathrm{RCONH}\overset{OH}{\underset{R}{C}}\cdot + CO_2$ followed by (b)  $\mathrm{RCONH}\overset{OH}{\underset{R}{C}}\cdot$ $+ Fe(II) + H_2O \longrightarrow RCONH_2 + RCHO + Fe(III) + OH^-$.

19. The rate constants for reaction of N-acetylalanine and Fe(II) are both about $\sim 2\times10^8\ M^{-1}\ sec^{-1}$. See Refs. 9, 20.

20. M. Anbar and P. Neta, Intern. J. Appl. Radiation Isotopes <u>17</u>, 493 (1967).

21. Cf. T. Shiga, J. Phys. Chem. <u>69</u>, 3805 (1965).

Table 1. Product yields in the γ-radiolysis of 0.05 M N-acetylalanine, O_2-saturated, pH 3.

Product	Yield (G)[a]
NH_3	2.9
CH_3COOH	2.8
H_2O_2	2.2
(ROOH)	≃0.5[b]
$CH_3COCOOH$ CH_3CHO	≤0.2

[a] Product yields are independent of dose up to $\sim 2\times10^{19}$ eV/ml.

[b] Unspecified.

FIGURE CAPTIONS

Fig. 1. Effect of solute concentration in the γ-radiolysis of N-acetylalanine in O_2-saturated solution; pH 7, 0; pH 3, ●. Dose = 1.5×10^{19} eV/ml.

Fig. 2. Effect of Fe(II) in the γ-radiolysis of 0.05 M N-acetylalanine, O_2-saturated, pH 3; $G(NH_3)$, ●; $G(CH_3COCOOH)$, ⊕. At 10^{-3} M Fe(II), $G(NH_3) = G(CH_3COCOOH) + G(CH_3CHO) \simeq 7.6$. These yields are strongly dose-dependent and fall off rapidly at values above $\sim 0.5 \times 10^{18}$ ev/ml.

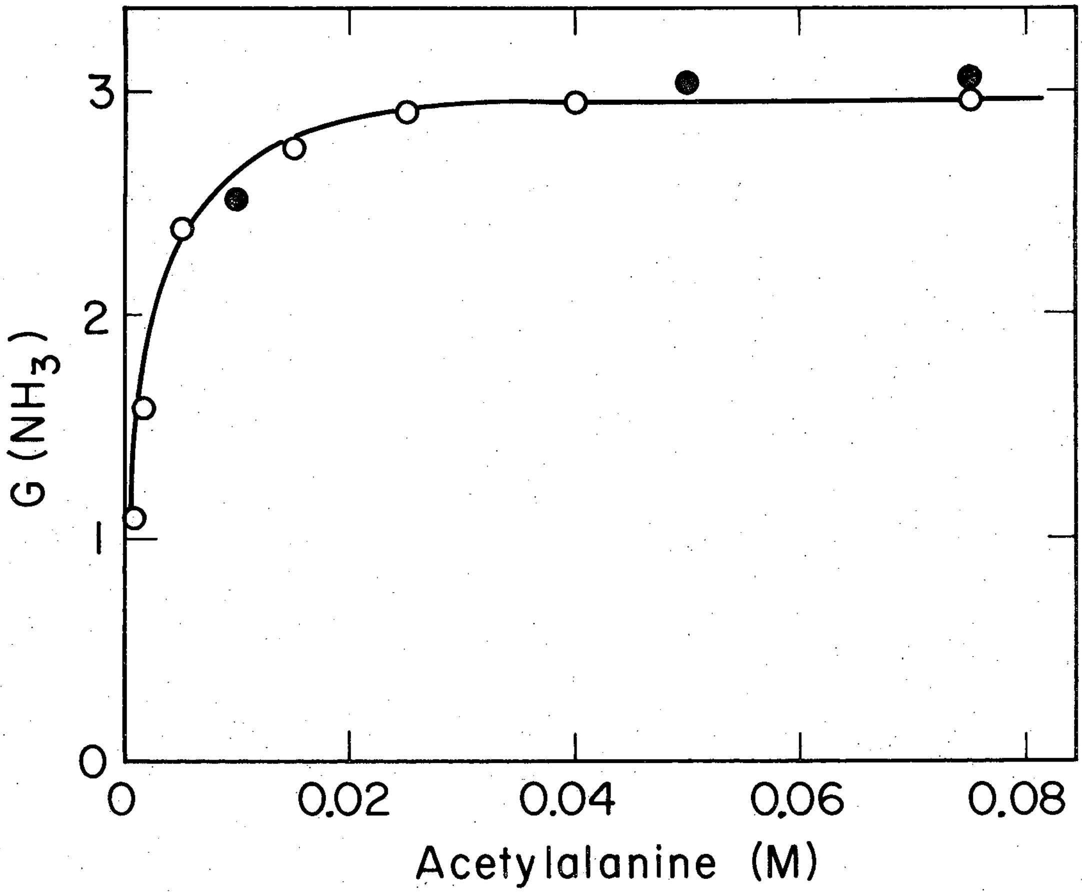


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Fig. 1

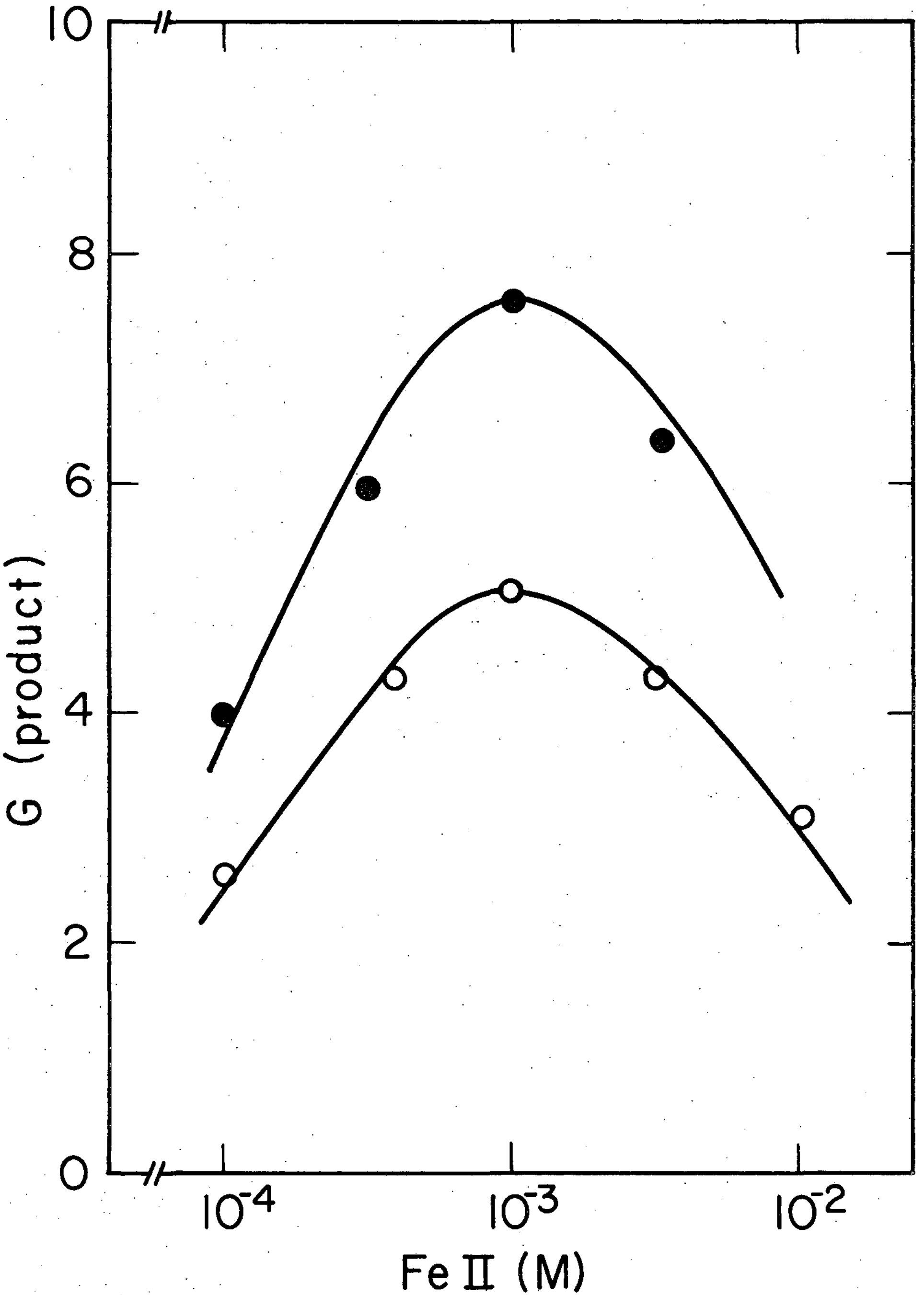

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Fig. 2

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