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The radiation-chemistry of simple peptides such as N-acetylglycine and N-acetylalanine in dilute aqueous solution can be interpreted almost exclusively in terms of the formation and subsequent reaction of the peptide radicals $RCONH\dot{C}R_2$.[1,2] In neutral solutions such radicals are formed predominantly through OH attack

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

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

where reaction 1 represents the radiation-induced step.[3-5] In the absence of oxygen, the reaction

$$2\ RCONH\dot{C}R_2 \rightarrow \begin{matrix} RCONHCR_2 \\ | \\ RCONHCR_2 \end{matrix} \qquad (3)$$

leads to the formation of the α,α' diamino succinic acid derivative. A fraction of the $RCONH\dot{C}R_2$ radicals undergo further oxidation through reactions of the

type

$$2\ RCONH\dot{C}R_2 \rightarrow RCON{=}CR_2 + RCONHCHR_2 \qquad (4)$$

$$H_2O_2 + RCONH\dot{C}R_2 \rightarrow RCONHC(OH)R_2 + OH \qquad (5)$$

where the H_2O_2 in reaction 5 is derived from the radiation-induced step 1. The products of reactions 4,5 are labile and readily decompose on mild hydrolysis e.g.

$$H_2O + RCON{=}CR_2 \rightarrow RCOOH + NH_3 + R_2CO \qquad (6)$$

to yield ammonia and carbonyl products. In the γ-radiolysis of evacuated .05M acetylalanine solutions at pH 7, $G(NH_3) \simeq G(>CO) \simeq 0.5$.

We find, however, that there is a very marked increase in $G(NH_3)$ as the concentration of the acetylalanine is increased above 0.1M. Data[6] obtained in the γ-radiolysis of O_2-free solutions of acetylalanine are given in Fig. 1. The ammonia yield approaches a limiting value of $G(NH_3) = 3$ in the concentration range 2M to 3M. This increase in $G(NH_3)$ is not accompanied by a corresponding increase in the yield of carbonyl products; $G(>CO) \sim 0.7$ over the entire concentration range 0.1M to 3M. Hence, the increase in $G(NH_3)$ cannot be explained in terms of an enhancement in the yields of reactions 2,4.[5] In fact, the increase in $G(NH_3)$ does not appear to be related in any significant way to the reactivity of the OH radical or its precursor H_2O[+7]. We find, for example, that addition of formate ion, which is an effective OH scavenger,

$$OH + HCOO^- \rightarrow H_2O + COO^- \qquad (7)$$

($k_7 = 2.5 \times 10^9 M^{-1}sec^{-1}$)[8] at concentrations as high as 0.75M has essentially no effect on $G(NH_3)$ from 2M acetylalanine ($k_2 = 2 \times 10^8 M^{-1}sec^{-1}$)[9]. Negative results were also obtained with phenol as the competing OH scavenger.

The evidence is that a quite different reaction mode sets in at acetylalanine concentrations above 0.1M. Chemical analysis[6] of the irradiated solutions reveal that propionic acid which is produced in negligible yield ($G \leq 0.1$) in .05M acetylalanine solution becomes a major product at the higher solute concentrations; in 2M acetylalanine the propionic acid yield corresponds to $G = 1.6$.[10] The possibility that amide and propionic acid arise as a consequence of reaction of the type

$$e^-_{aq} + RCONHCHR_2 \rightarrow (RCONHCHR_2)^- \qquad (8)$$

$$(RCONHCHR_2)^- \rightarrow RCONH^- + \dot{C}HR_2 \qquad (9)$$

must be considered; we have shown elsewhere that $k_8 = 1 \times 10^7$ for acetylalanine at pH 7.[11] However, addition of chloracetate ion which is an effective electron scavenger

$$e^-_{aq} + RCl \rightarrow R + Cl^- \qquad (10)$$

does not significantly reduce $G(NH_3)$ from 2M acetylalanine even at chloracetate concentrations as high as .05M; under this condition $G(NH_3) = 2.3$, $G(Cl^-) = 2.5$.[12] Additional evidence against reaction 9 is the finding that $G(NH_3)$ from the 2M solution at pH 7 remains essentially constant on increasing the acidity to pH 1.

($k_{H^+ + e^-_{aq}} = 2 \times 10^{10}$).[8] We must conclude then that the removal of OH, e^-_{aq} (and also of H_2O^+ and e^-) is not involved in the radiolytic degradation of the peptide bond in these concentrated solutions.

Now, certain compounds such as benzophenone and naphthalene, for example, react rapidly with e^-_{aq} and OH[8] and also have the additional property of being efficient quenchers of excited states.[13] We find that addition of naphthalene sulfonic acid in millimolar concentration effects a sharp decrease in $G(NH_3)$ from 2M acetylalanine as shown in Fig. 1. The reciprocal yield plot (Fig. 1 insert) extrapolates to give G = 1.6 as the limiting yield for production of species which the present evidence suggests are excited states of acetylalanine.

The mechanism for formation of $RCONHCHR_2^{\ddagger}$ has not been conclusively established. However, we have recently found that the propionic acid yield which approaches G = 1.6 in 2M acetylalanine does not increase further with increasing solute concentration to 10M. In fact, this same yield is obtained in the γ-radiolysis of acetylalanine in the polycrystalline form.[14] Our present conclusion is (a) that preferential excitation by low-energy electrons[15] is involved e.g.

$$\bar{e} + RCONHCHR_2 \rightarrow RCONHCHR_2^{\ddagger} + e^- \qquad (11)$$

and (b) that the species $RCONHCHR_2^{\ddagger}$ are removed through reaction of the type

$$RCONHCHR_2^{\ddagger} + RCONHCHR_2 \rightarrow RCONH\dot{C}R_2 + RCONH_2 + \dot{C}HR_2 \qquad (12)$$

Current work is expected to provide detailed information on the physical and chemical properties of the species $RCONHCHR_2^{\ddagger}$.

REFERENCES AND FOOTNOTES

*This work was done under the auspices of the U. S. Atomic Energy Commission.

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5. The relatively small but apparently very real discrepancies in the reported 100 ev yields of the products of reaction 1 have been discussed by Allen (ref. 3). Recent measurements by Hochanadel and Casey (ref. 4) give $G_{OH} = 2.59$, $G_{e^-_{aq}} = 2.58$, $G_H = 0.55$, $G_{H_2} = 0.45$, $G_{H_2O_2} = 0.72$.

6. Experimental methods employed in the present work are described in refs. 1 and 2. The vapor-phase chromatography was performed by Mr. H. A. Sokol.

7. The apparent yield of reaction 2 is independent of solute concentration i.e., $G(RCONH\dot{C}R_2) = 2.5 \simeq G_{OH}$ in both dilute and concentrated solution (ref. 2). However, since OH arises from H_2O^+ via $H_2O^+ + H_2O \rightarrow H_3O^+ + OH$ (ref. 3b), we cannot rule out the possibility that at the higher solute concentrations a fraction of the H_2O^+ species reacts directly with $RCONHCHR_2$ to give $RCONH\dot{C}R_2$.

8. For a recent compilation of rate data see M. Anbar and P. Neta, Int. J. appl. Radiat. Isotopes 17, 493 (1967).

9. Determined through measurements of competition kinetics in solutions of p-nitrosodimethylaraline after the method of I. Kralic and C. N. Trumbore, J. Amer. Chem. Soc. 87, 2547 (1965).

10. The propionic acid yield is strongly dependent on dose. The value G(propionic) = 1.6 represents the initial yield at a dose of 2×10^{18} ev/gm.

11. R. L. S. Willix and W. M. Garrison UCRL-17285 (1966) Radiation Res. In Press.

12. The fact that the electron can be quantitatively measured as chloride ion in this system also rules out the possibility that reactions 8,9 occur prior to the hydration of e^-.

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FIGURE CAPTION

Fig. 1. Amide-ammonia yield, $G(NH_3)$, as a function of acetylalanine concentration in oxygen-free solution at pH 7 under γ-rays. Insert: effect of naphthalene sulfonate on $G(NH_3)$ from a 2M acetylalanine solution.

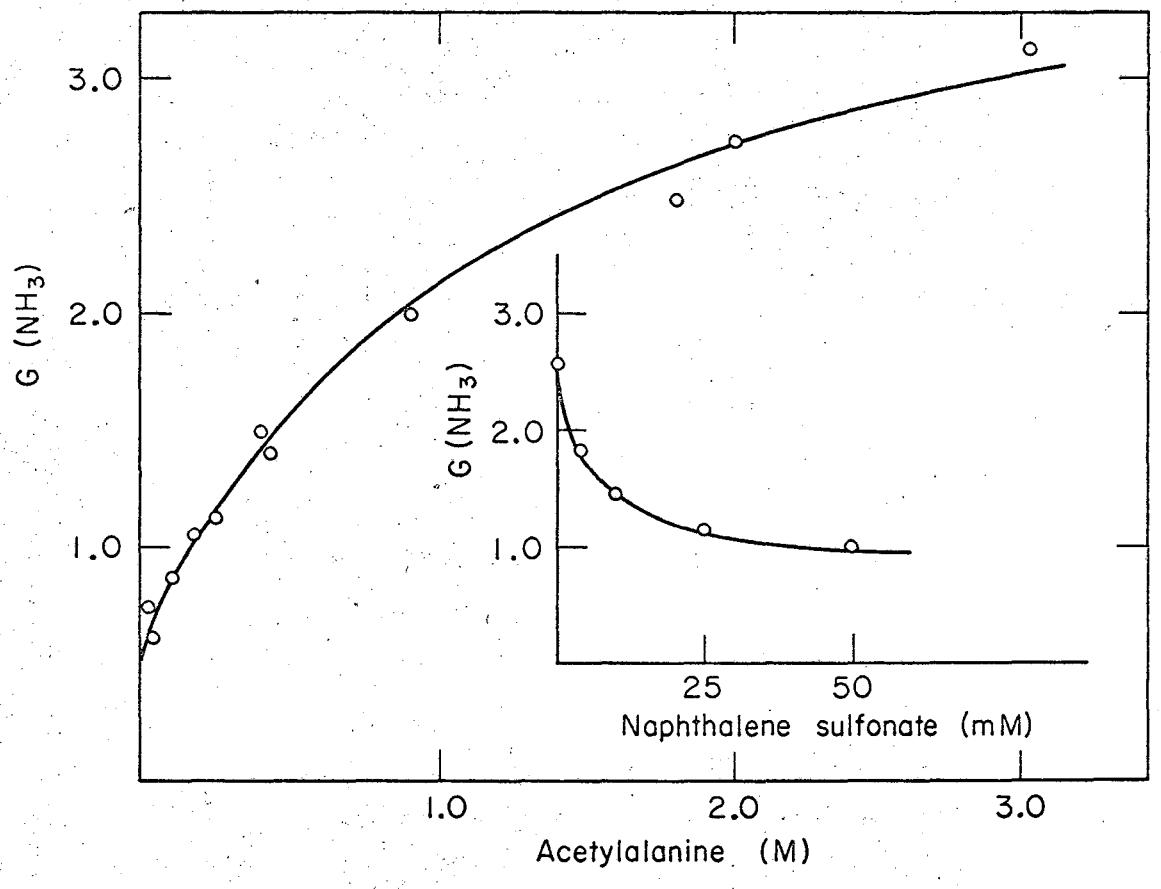

Figure 1

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