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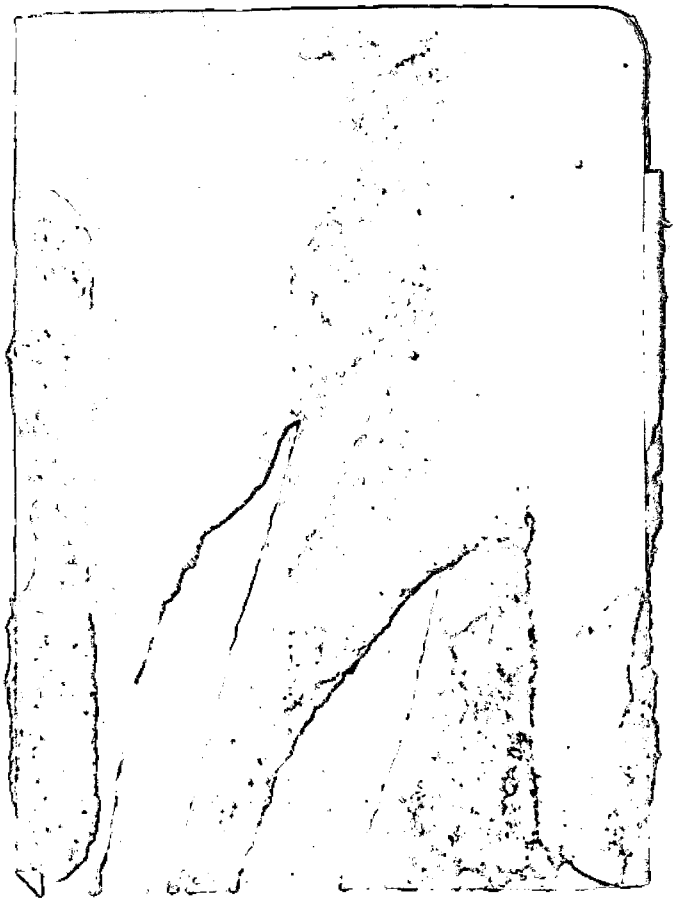

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UCRL-18849

ROLE OF EXCITED SPECIES $(RCONHCHR_2)^*$ IN THE RADIOLYTIC OXIDATION OF THE PEPTIDE MAIN-CHAIN IN AQUEOUS SYSTEMS[1]

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Studies of the radiation chemistry of simple peptides in dilute oxygen-free solutions have shown[2] that the labile products of the γ-radiolysis of water[3,4]

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

undergo reactions of the type

$$e^-_{aq} + RCONHCHR_2 + H_2O \longrightarrow \dot{R}C(OH)NHCHR_2 + OH^- \qquad (2)$$

$$OH(H) + RCONHCHR_2 \longrightarrow RCONH\dot{C}R_2 + H_2O(H_2) \qquad (3)$$

Subsequent interactions do not lead to degradation of the peptide main-chain in any appreciable yield.[2,5] The small amounts of "amide-like" products formed in the γ-radiolysis of dilute oxygen-free solutions of N-acetylglycine and N-acetylalanine are attributed to the over-all reaction[2]

$$RCONH\dot{C}R_2 + H_2O_2 \longrightarrow RCONH_2 + R_2CO + OH \qquad (4)$$

which yields $G(NH_3) \simeq G(R_2CO) \simeq 0.5$ on hydrolysis.

However, we have recently found that as the peptide concentration in these oxygen-free solutions is increased above ~ 0.1 M, an abrupt increase in the amide-ammonia yield is observed.[5] For example, in the γ-radiolysis of N-acetylalanine solutions the amide yield, expressed in terms of ammonia released on hydrolysis,[6] increases from $G(NH_3) \simeq 0.5$ in 0.1 M solution to $G(NH_3) \simeq 2$ in 1 M solution. The yield then tends to level off at $G(NH_3) \simeq 3$ in the concentration range 2 M to 3 M. Propionic acid is the principal concomitant product associated with this enhancement in the amide yield. The chemical evidence in toto[5,7,8] is that a major fraction of this "extra" chemistry is derived from excited-molecule reactions

$$e^- + RCONHCHR_2 \longrightarrow (RCONHCHR_2)^* + e^- \qquad (5)$$

$$(RCONHCHR_2)^* + RCONHCHR_2 \longrightarrow \dot{R}CONHCR_2 + RCONH_2 + \dot{C}HR_2 \qquad (6)$$

where e^- of reaction (5) represents a low-energy (subexcitation) electron.[9]

Now, in an earlier publication[10] on the radiolytic oxidation of N-acetyl derivatives of glycine and alanine in aqueous solution containing FeIII as the oxidizing scavenger, we found that the observed chemistry at peptide concentrations ranging from .05 M to 2 M could be interpreted exclusively in terms of the reported yields of reaction (1). For example, in the γ-radiolysis of 0.1 M solutions of N-acetylalanine containing .05 M FeIII to preferentially scavenge e^-_{aq} and H, the $\dot{R}CONHCR_2$ radicals formed through OH attack are quantitatively oxidized in accord with the over-all chemistry

$$RCONHCR_2 + FeIII + H_2O \longrightarrow RCONH_2 + R_2CO + FeII + H^+ \qquad (7)$$

to give[6,11] $G(NH_3) \simeq G(R_2CO) = 3.2 \simeq G_{OH} + G_{H_2O_2}$ where R_2CO represents the keto acid; the aldehyde yield is nil. With increasing N-acetylalanine concentration the degradation yield increases somewhat but levels off at[12] $G(NH_3) \simeq G(R_2CO) = 3.9 \simeq G_{OH} + G_{H_2O_2} + G_H$ in the concentration range 0.5 M to 2 M.

If oxygen is used as the oxidizing scavenger the over-all chemistry is more complicated but the peptide radicals in this case also are quantitatively degraded in accord with the stoichiometry[10]

$$RCONHCR_2 + O_2 + H_2O \longrightarrow RCONH_2 + \text{products} \qquad (8)$$

In the γ-radiolysis of oxygen-saturated 0.1 M solutions of N-acetylglycine and N-acetylalanine we have observed[6,10] $G(NH_3) \simeq 2.9 \simeq G_{OH}$. And we now find (Fig. 1) that on increasing the concentration of N-acetylalanine to 2 M and above in oxygen-saturated solution the increase in $G(NH_3)$ is small and in agreement with the stoichiometry[12] $G(NH_3) \simeq 3.4 \simeq G_{OH} + G_H$. The aldehyde yield is low.[13] The keto acid yield is nil.[14] Apparently the major nitrogen-free organic products of reaction (8) are of a higher order of oxidation.[10]

Our concern here is with the fact that in the presence of oxidizing solutes such as O_2 and FeIII there appears to be no contribution of the excited species $(RCONHCHR_2)^*$ which we have estimated[5,7] to be produced with $G \geq 1.9$ for a 2 M N-acetylalanine solution in the absence of oxidizing solutes. There are at least two alternate explanations for this seeming discrepancy

(a) O_2 and FeIII quench $(RCONHCHR_2)^*$ through physical processes as do the excitation scavengers referred to above[5,7,8] (b) O_2 and FeIII react with $(RCONHCHR_2)^*$ to yield heretofore undetected products.

We conclude that the latter is the case since hot acid-hydrolysis[15] of the irradiated N-acetylalanine solutions prior to assay leads to a marked increase in $G(NH_3)$ which increase is accompanied by the production of acetaldehyde in the relationship $\Delta G(NH_3) \simeq \Delta G(RCHO)$ as shown in Fig. 1.

These results are both quantitatively and qualitatively consistent with the concept that $(RCONHCHR_2)^*$ is oxidized by both O_2 and FeIII to yield the dehydropeptide derivative RCON=CHR and that this species requires a prior acid hydrolysis to release ammonia and aldehyde

$$RCON{=}CHR + 2H_2O \longrightarrow RCOOH + NH_3 + RCHO \qquad (9)$$

which are then detected by the analytical procedures used in the present study.[6,13]

It is also of interest to note (Fig. 1) that the full aldehyde yield can be measured directly (without the prior hydrolysis in hot 0.2 N sulfuric acid) if the procedure of Johnson and Scholes[16] is used. This procedure employs high concentrations of 2,4-dinitrophenylhydrazine (2,4-DNPH) in 60 percent perchloric acid as solvent. Under these conditions products of the type RCON=CHR either undergo hydrolysis in the cold or react directly with the 2,4-DNPH reagent to yield the hydrazone derivative.

A full report of the radiation chemistry of the peptide main-chain in concentrated aqueous solutions is in preparation.

FOOTNOTES AND REFERENCES

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

(2) (a) W. M. Garrison, Radiation Res., Suppl. 4, 158, (1964); (b) R. L. S. Willix and W. M. Garrison, Radiation Res., 32, 452 (1967); (c) J. Holian and W. M. Garrison, J. Phys. Chem., 72, 4721 (1968).

(3) C. J. Hochanadel and R. Casey, Radiation Res., 25, 198 (1965) report $G_{OH} = 2.59$, $G_{e^-_{aq}} = 2.58$, $G_H = 0.55$, $G_{H_2} = 0.45$, $G_{H_2O_2} = 0.72$ as the 100 ev yields for reaction (1). The maximal OH yield at high solute concentrations appears to be $G_{OH} \simeq 2.9$ (Ref. 4).

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

(5) M. A. J. Rodgers and W. M. Garrison, J. Phys. Chem., 72, 758 (1968).

(6) Hydrolysis here was done in 2 N 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 hrs. (Ref. 10).

(7) W. M. Garrison, M. E. Jayko, M. A. J. Rodgers, H. A. Sokol and W. Bennett-Corniea, Adv. Chem. Ser., 81, 384 (1968).

(8) Quantitative scavenging of e^-_{aq} and OH respectively by scavengers such as chloracetate and formate ions, for example, has essentially no effect on this amide yield. However, certain excitation scavengers such as napthalene sulfonic acid are effective in quenching amide production (Ref. 5,7).

(9) R. L. Platzman, Radiation Res., 2 1, (1955).

(10) H. L. Atkins, W. Bennett-Corniea and W. M. Garrison, J. Phys. Chem., 71, 772 (1967).

(11) Hydrogen peroxide formed in reaction (1) yields additional OH via

$$FeII + H_2O_2 \longrightarrow FeIII + OH^- + OH.$$

(12) The peptide at the higher concentrations competes with the oxidizing scavenger for H via reaction (3).

(13) We use here the method of E. Sawicki et al., Anal. Chem., 33, 93 (1961). The 3-methyl-2-benzothiazolone hydrazine reagent (MBTH) is specific for aliphatic aldehydes, RCHO.

(14) The 2,4-dinitrophenylhydrazine reagent (2,4-DNPH) at low concentration (2 to 3 mole-excess) in dilute (0.1 N) acid may be used in the determination of α-keto acids without aldehyde interference. H. A. Sokol, to be published.

(15) Solutions were hydrolyzed under nitrogen in 0.2 N sulfuric acid at 95°C for 1 hr. Prior to hydrolysis the FeIII was removed with Dowex 50 (acid form). The oxygenated solutions received a prior treatment with platinum black to remove hydrogen peroxide, cf. W. M. Garrison, M. E. Jayko, W. Bennett, Radiation Res., 16, 483 (1962).

(16) G. R. A. Johnson and G. Scholes, Ind. Eng. Chem. Anal. Ed., 79, 217 (1954).

FIGURE CAPTIONS

Fig. 1. Product yields in the γ-radiolysis of concentrated solutions of N-acetylalanine. [O_2-saturated], G(RCHO) by MBTH method[13] before, (○) and after, (⊕) acid hydrolysis;[15] G(RCHO) by direct application of Johnson-Scholes method,[16] (◑); G(NH_3) after amide hydrolysis in base,[6] (□); G(NH_3) after acid hydrolysis,[15] (■). [.05 M FeIII] G(RCHO) by MBTH method after acid hydrolysis, (●); G(RCHO) by direct application of Johnson-Scholes method, (◐); G(NH_3) after acid hydrolysis, (▲).

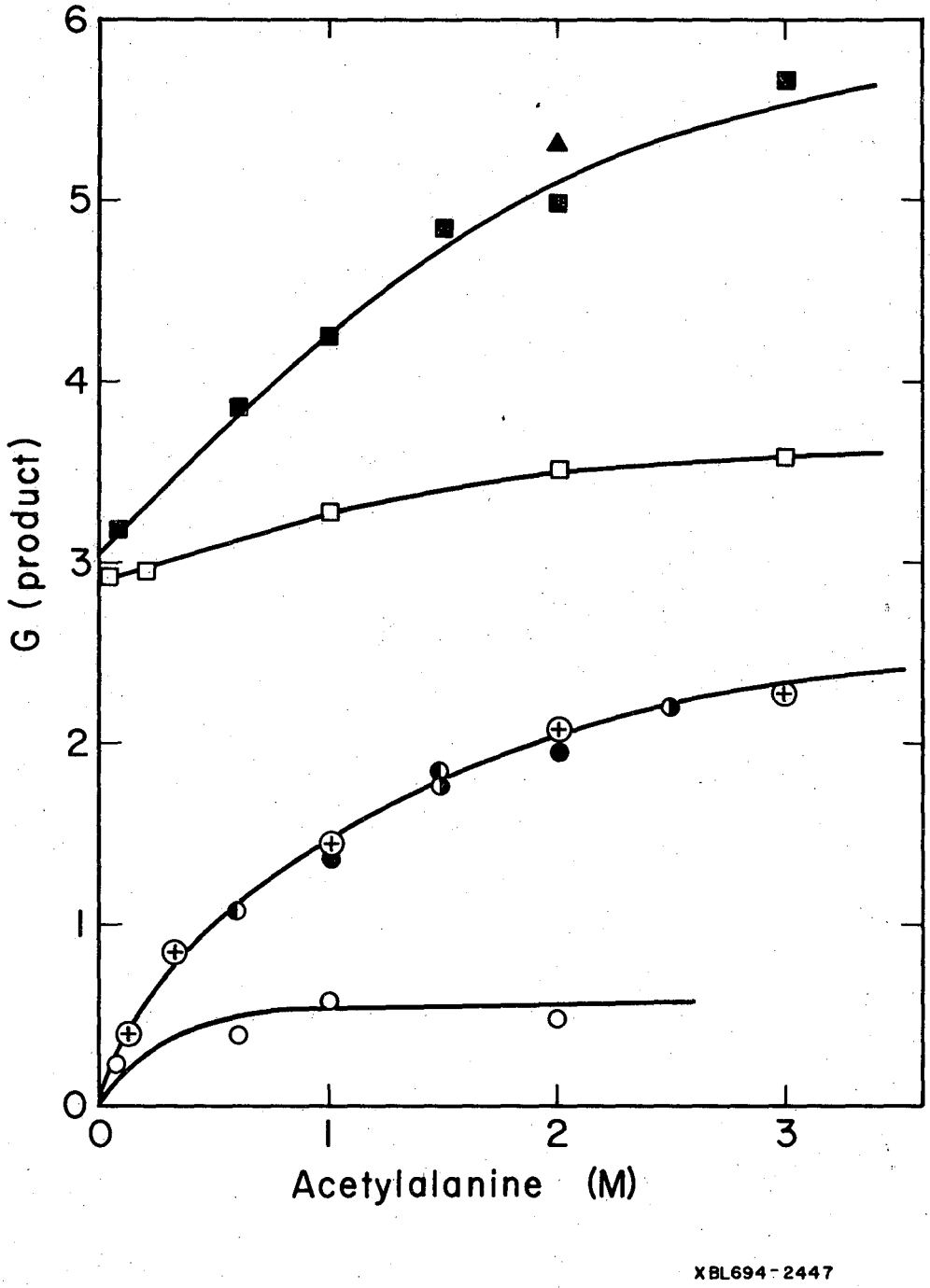

Fig. 1.

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