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Phys. Chem.

UCRL-17004

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AEC Contract No. W-7405-eng-48

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THE RADIATION-INDUCED OXIDATION OF PEPTIDES IN AQUEOUS SOLUTION[1]

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Radiolysis of compounds of the type $RCONHCHR_2$ in dilute aqueous solution containing O_2 leads to formation of labile peptide derivatives which are readily degraded on mild hydrolysis to give ammonia and carbonyl compounds as characteristic products.[2] It has been proposed[2,3] that such oxidation in the case of peptides derived from the simpler amino acids, glycine and alanine, is initiated by a preferential attack of the OH radical at the carbon-hydrogen position α to the nitrogen function. The over-all reaction scheme includes the radiation-induced step[4]

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

followed by

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

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

The reducing species e^-_{aq} and H are scavenged quantitatively by O_2 to form O_2^- and HO_2 which are related by the equilibrium[5] $HO_2 \rightleftharpoons O_2^- + H^+$. Proposed reaction modes for removal of the intermediate peroxy radicals include

$$RCONHC(O_2)R_2 \longrightarrow RCON{=}CR_2 + HO_2 \quad (4)$$

$$HO_2 + RCONHC(O_2)R_2 \longrightarrow RCONHC(OOH)R_2 + O_2 \quad (5)$$

$$H_2O + RCONHC(OOH)R_2 \longrightarrow RCONHC(OH)R_2 + H_2O_2 \quad (6)$$

where $RCON{=}CR_2$ represents a dehydropeptide and $RCONHC(OH)R_2$ the corresponding hydrate. Such compounds readily decompose on hydrolysis[6]

$$RCON{=}CR_2 + 2H_2O \longrightarrow RCOOH + NH_3 + R_2CO \quad (7)$$

$$RCONHC(OH)R_2 + H_2O \longrightarrow RCOOH + NH_3 + R_2CO \quad (8)$$

The above reaction scheme requires that the ammonia and carbonyl yields be in the relationship $G(NH_3) = G(R_2CO) = G_{OH}$ where the latter term represents the 100 eV yield for OH production in the radiation induced step, 1. We have measured ammonia and carbonyl yields in the γ-ray induced oxidation of N-acetylglycine,[3c] glycine anhydride, N-acetylalanine and alanine anhydride in oxygenated 0.1 M solution and we find for each system, $G(NH_3) \simeq 3$ which value is consistent with recent measurements[7] of the maximal yield for the production of OH radicals in water under γ-rays. On the other hand, we also find that carbonyl production in these simple peptide systems is not in accord with the quantitative requirements of the proposed oxidation scheme; the initial carbonyl yields are uniformly low with $G(R_2CO) \leq 0.8$. There is then the question as to whether this apparent discrepancy arises from a) an incorrect formulation of the locus of initial OH attack or from b) unspecified complexities in the chemistry of removal of the peroxy radicals $RCONHC(O_2)R_2$.

To obtain specific information on this point, we have employed FeIII instead of O_2 as the scavenger of intermediate radicals formed in the radiolysis of N-acetylalanine and N-acetylglycine. Heavy metal ions such as FeIII and CuII oxidize organic free radicals in aqueous solution by electron transfer and by ligand transfer.[8] Such reactions in the case of the peptide radical $RCONH\dot{C}R_2$ would correspond to:

$$RCONH\dot{C}R_2 + FeIII \longrightarrow RCON{=}CR_2 + FeII + H^+ \qquad (9)$$

$$RCONH\dot{C}R_2 + FeIII + H_2O \longrightarrow RCONHC(OH)R_2 + FeII + H^+ \qquad (10)$$

respectively. We note that the organic products of reactions 9, 10 are identical with the postulated products of reactions 4, 6.

Experimental

Solutions containing the acetylamino acid (Nutritional Biochemicals, twice recrystallized) plus ferric sulfate (C.P.) were adjusted to the desired pH with sulfuric acid, evacuated, and then irradiated with Co^{60} γ-rays to a dose of not more than ~5×10^{18} eV/ml. Above this value the dose-yield curves were no longer linear. Dose was determined with the Fricke dosimeter, $\epsilon_{FeIII} = 2130$ at 305 mμ and 22°C. Immediately after irradiation, an aliquot was passed through a Dowex 50 column (1 cm × 10 cm), acid form, to remove iron. The eluant was made 2 N in hydrochloric acid, heated to 100°C for 60 minutes, cooled, and assayed for α-keto acid and aldehyde through use of the 2,4-dinitrophenylhydrazine reagent.[9] A second aliquot was made 2 N in sodium hydroxide and allowed to stand 24 hours in the outer compartment of a Conway diffusion

cell to liberate ammonia which was collected in 0.1 N sulfuric acid in the inner compartment, and assayed by the standard Tollen procedure. Control runs established that product hydrolysis and ammonia transfer were quantitative under these experimental conditions.

Results and Discussion

Ammonia production (Fig. 1) in 0.1 M acetylalanine increases abruptly from $G(NH_3) = 0.7$ to $G(NH_3) = 4.3$ with increasing concentrations of FeIII up to $\sim 10^{-3}$ M. The ammonia yield then falls gradually to a limiting value of $G(NH_3) = 3.3$ at the higher FeIII concentrations. We also find under these conditions that pyruvic acid and ammonia are formed in equal molar yields. Yields of glyoxylic acid and ammonia from acetylglycine also show this same quantitative relationship. Aldehyde yields from these systems are low, $G \simeq 0.1$.

At the higher (FeIII)/(peptide) ratios, the reducing species e^-_{aq} and H are preferentially scavenged by FeIII and the yield for peptide oxidation through OH attack is in accord with

$$-G(\text{peptide}) = G(NH_3) = G(RCOCOOH) \simeq 3.2 \simeq G_{OH} + G_{H_2O_2} \quad .$$

Hydrogen peroxide formed in the radiation-induced step, 1, reacts rapidly with FeII to give an additional yield of OH radicals[10]

$$\text{FeII} + H_2O_2 \longrightarrow \text{FeIII} + OH^- + OH \qquad (11)$$

The maximum in the yield curve shown in Fig. 1 is attributed to the onset of the reaction

$$H + RCONHCHR_2 \longrightarrow RCONH\dot{C}R_2 + H_2 \qquad (12)$$

in competition with

$$H + FeIII \longrightarrow FeII + H^+ \qquad (13)$$

at the lower (FeIII)/(peptide) ratios. The $RCONH\dot{C}R_2$ radicals from both reactions 2 and 12 are then available for oxidation by FeIII. This effect is shown more clearly in Fig. 2 which gives ammonia and pyruvic acid yields as a function of acetylalanine concentration over the range 10^{-3} M to 1.5 M, in the presence of 0.05 M FeIII. The limiting value for peptide oxidation at the higher acetylalanine concentrations is given by

$$-G(\text{peptide}) = G(NH_3) = G(R_2CO) \simeq 4.0 \simeq G_{OH} + G_{H_2O_2} + G_H$$

We conclude that the reaction of OH and H radicals with these peptide derivatives of the simpler amino acids, glycine and alanine, occurs essentially quantitatively at the α-position as formulated in reactions 2,12.[11] The present data also establish the quantitative oxidation of $RCONH\dot{C}R_2$ radicals by FeIII via reactions 9, 10. In the case of acetylalanine such oxidation appears to occur almost exclusively through ligand transfer (reaction 10) since measurements of the optical absorption of the irradiated solutions (after removal of FeIII) reveal negligible absorption above 230 mμ when read differentially against

unirradiated control solution. Absorption by control solutions containing authentic acetyldehydroalanine[12] (ϵ_{240} = 6050) show that G values of >0.1 for reaction 9 would be detectable. To our knowledge the optical properties of acetyldehydroglycine have not been described.

The low carbonyl yields obtained when O_2 is used in place of FeIII as the radical scavenger are interpreted here as evidence that other more complex degradation reactions occur in parallel with the dehydrogenation and hydroxylation reactions, 4-6. The nature of these more complex branching reactions of the peroxy radical $RCONHC(O_2)R_2$ is presently under study.

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

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(12) We are indebted to Dr. B. M. Weeks for the sample of authentic acetyldehydroalanine which was prepared after the method of V. E. Price and J. P. Greenstein. Arch. Biochem. 10, 383 (1948).

Legends for Figures

Fig. 1. Effect of FeIII concentration on the yields of ammonia (●) and pyruvic acid (▲) from 0.1 M acetylalanine and of ammonia (O) and glyoxylic acid (△) from 0.1 M acetylglycine.

Fig. 2. Effect of acetylalanine concentration on yields of ammonia (●) and of pyruvic acid (▲) from solutions containing 0.05 M FeIII.

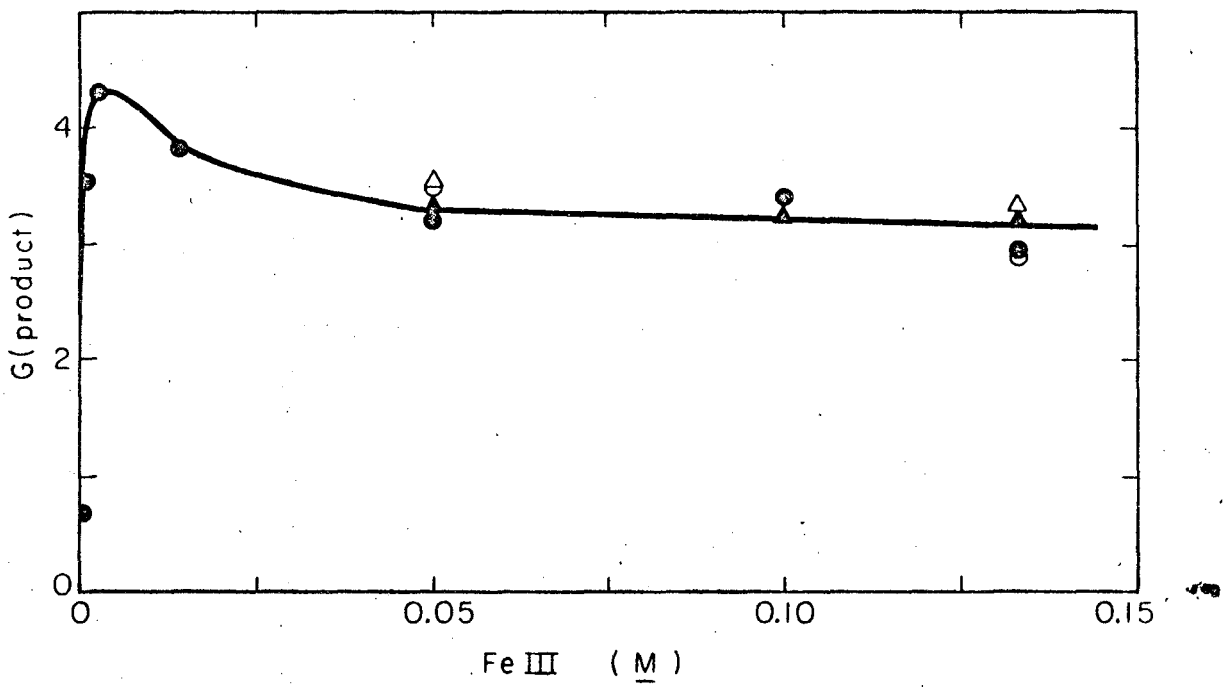

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Fig. 1

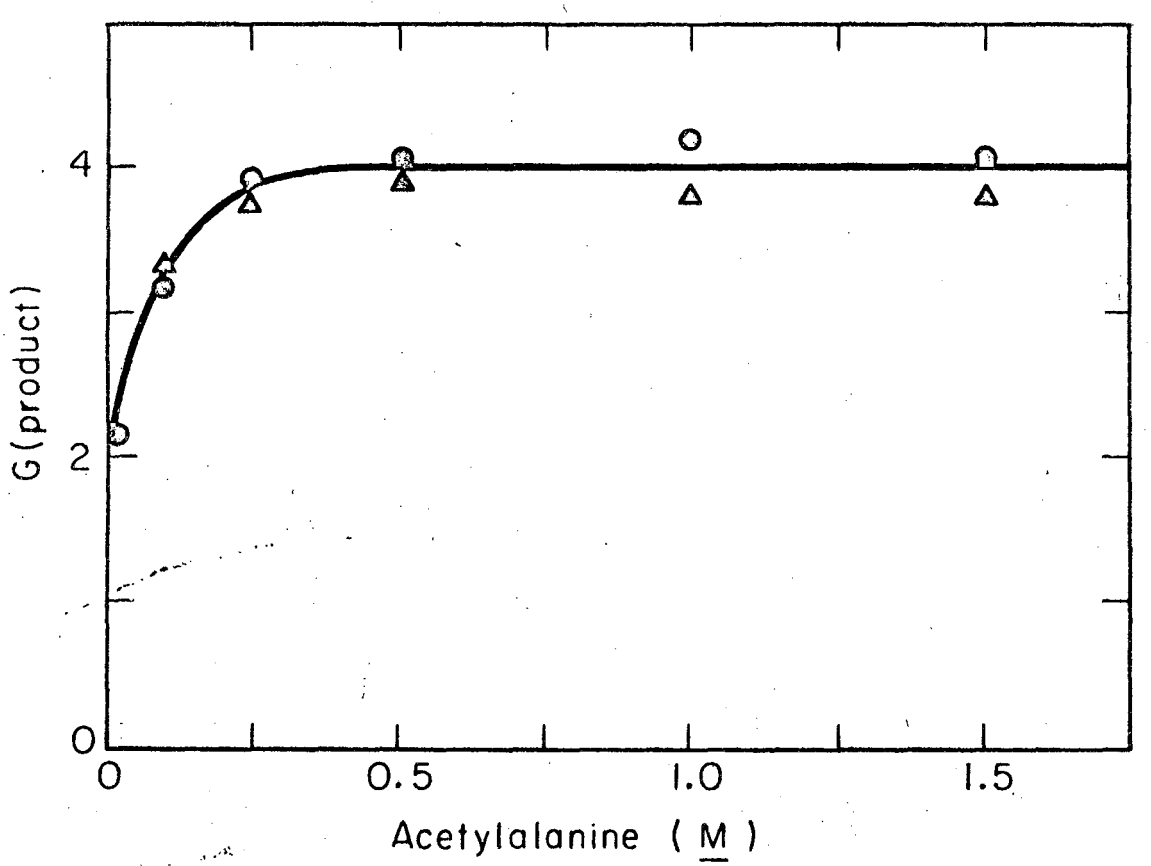

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Fig. 2

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