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RADIOLYTIC CLEAVAGE OF THE PEPTIDE MAIN-CHAIN IN CONCENTRATED AQUEOUS SOLUTION: ENERGY LEVEL OF EXCITED-MOLECULE INTERMEDIATES[1]

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The γ-radiolysis of oxygen-free solutions of simple peptides such as N-acetylglycine and N-acetylalanine at concentrations above 0.1 M leads to formation of excited species $RCONHCHR_2^*$. Reactions of these excited-molecule intermediates lead to cleavage of the peptide main-chain with formation of amide and fatty acid functions. Through use of a series of aromatic second-solutes as excitation quenchers it is possible to assign the energy level of $RCONHCHR_2^*$ at ∿3.5 eV.

In the γ-radiolysis of dilute aqueous solutions, the observed chemical changes arise through reactions of solute with the hydrated electron and the radicals OH and H which are produced in the radiation-induced decomposition of solvent water (1-4).[3]

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

Studies of the radiation chemistry of simple peptides such as N-acetylglycine and N-acetylalanine in dilute oxygen-free solution have shown that labile species e^-_{aq}, OH, and H are preferentially removed through reaction at the peptide main chain. Such reactions do not, however, lead to main-chain cleavage in any appreciable yield (5-8). Small amounts of amide-like products are produced but the ammonia yield on mild hydrolysis is relatively low with $G(NH_3) \simeq 0.4$.

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

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[3]Bielski and Allen (4) report the following 100 eV yields (G values) for reaction 1: $G_{OH} = 2.74$, $G_{e^-_{aq}} = 2.76$, $G_H = 0.55$, $G_{H_2} = 0.40$, $G_{H_2O_2} = 1.00$.

We have also observed (7) that as the peptide concentration is increased above ∿0.1 M, the amide yield increases abruptly and approaches a limiting value of $G(NH_3) \approx 3$ in the concentration range 2M to 3M. We suggested in a preliminary report (7) that degradation of the peptide main-chain at high solute concentrations involves excited-molecule reactions. The present work extends the preliminary findings and provides experimental evidence that the excited level of N-acetylalanine corresponds to ∿3.5 eV.

Materials and Methods

The N-acetylalanine was obtained from Cyclo Chemical Corp. (NRC grade I) and was recrystallized twice from water. Water used in preparation of solutions was from a Barnstead still and was redistilled first from alkaline permanganate and then from phosphoric acid. The pH adjustments were made with sodium hydroxide.

Pyrex ampoules containing 10 ml of solution were de-gassed on the vacuum line and then sealed off with the torch. They were irradiated in a 10 kc ^{60}Co Gamma Cell (ambient temperature ∿35°C) at a dose-rate of 1×10^{18} eV/gm-min [$G(Fe^{3+} = 15.5$; $\varepsilon_{305} = 2180$ at 24°C].

Amide ammonia was determined after a modification of the method of Conway (9). Propionic acid was assayed by vapor-phase chromatography (10). Carbonyl products (pyruvic acid plus acetaldehyde) were determined after the method of Johnson and Scholes (11).

Results and Discussion

The radiation chemistry of N-acetylglycine and N-acetylalanine in dilute, neutral solution in the absence of O_2 may be represented (6,8,12) in terms of the radiation-induced step 1 followed by

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

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

The radical products of reactions 2,3 are subsequently removed mainly through the reconstitution reaction

$$H_2O + R\dot{C}(O^-)NHCHR + RCONH\dot{C}R \longrightarrow 2RCONHCHR_2 + OH^- \quad (4)$$

A fraction of the $RCONH\dot{C}R_2$ radicals undergo further oxidation of the type

$$2RCONH\dot{C}R_2 \longrightarrow RCON{=}CHR_2 + RCONHCHR_2 \quad (5)$$

$$H_2O_2 + RCONH\dot{C}R_2 \longrightarrow RCONHC(OH)R_2 + OH \quad (6)$$

where the H_2O_2 of reaction 6 is derived from the radiation-induced step 1. The oxidized products of reactions 5,6 are labile and readily decompose on mild hydrolysis

$$H_2O + RCON{=}CR_2 \longrightarrow RCONH_2 + R_2CO \quad (7)$$

$$RCONHC(OH)R_2 \longrightarrow RCONH_2 + R_2CO \quad (8)$$

$$H_2O + RCONH_2 \longrightarrow RCOOH + NH_3 \quad (9)$$

to give $G(NH_3) \simeq G(R_2CO) \simeq 0.4$.

Although reactions 1-9 provide an explanation of the radiation chemistry of the simpler N-acetyl amino acids in dilute solution, other processes become of major importance at higher solute concentrations. In the case of N-acetylalanine there is a very marked increase in the amide ammonia yield as the solute concentration is increased above 0.1 M as shown in Figure 1. We also see that the carbonyl yield is essentially independent of solute concentration over the range studied. Propionic acid is the principle concomitant product associated with the enhancement in the amide yield from N-acetylalanine.

The production of amide and fatty acid at the higher solute concentrations does not appear to be related in any significant way to the reactions of OH, H, e^-_{aq}. We have found (7) that addition of second-solutes at concentrations

sufficient to quantitatively scavenge the products of water radiolysis has relatively little effect on the amide yield in concentrated aqueous solution. The evidence is that a new reaction mode sets in at concentrations above 0.1 M. We have suggested (7) that such reaction is of the form

$$e^- + RCONHCHR_2 \longrightarrow RCONHCHR_2^* + e \qquad (10)$$

$$RCONHCHR_2^* + RCONHCHR_2 \longrightarrow RCONH\dot{C}R_2 + RCONH_2 + \dot{C}HR_2 \qquad (11)$$

Platzman (13) proposed some years ago that in the γ-radiolysis of concentrated aqueous solutions the solute may undergo electronic excitation through interaction with low-energy electrons as formulated in equation 10.

Now, aromatic compounds are, of course, known to be effective scavengers of excited states providing the energy level of the aromatic compound is lower than that of the excited species (14). We find experimentally that naphthalene sulfonic acid, benzoic acid and benzaldehyde are remarkably effective in quenching the formation of amide ammonia in 2M N-acetyalanine. Phenol and benzene sulfonic acid on the other hand are without effect even at the higher concentrations. Typical data are summarized in Figure 2.

The reason that certain aromatic compounds are effective quenchers and others are not becomes evident on examination of the energy-level diagram given in Figure 3. Data for the singlet and triplet levels of the aromatic compounds are taken from Calvert and Pitts (15). The value of the singlet level of N-acetylalanine is from the work of Saidel (16). We note first that although all of the aromatic solutes studied here have singlet levels below the peptide upper singlet all are not effective quenchers as we have noted. The correlation appears when the triplet levels are considered. We see that those compounds that are effective quenchers viz., benzaldehyde, naphthalene sulfonic acid, and benzoic acid have the lower triplet levels as compared to phenol and benzene sulfonic acid. The change from quenching to nonquenching occurs between benzoic acid (27,200 cm^{-1}) and phenol (28,500 cm^{-1}). In other words

the energy of the excited state, $RCONHCHR_2^*$, in the case of N-acetylalanine must be between these two values, at ≈28,000 cm^{-1} ≈ 80 kcal ≈ 3.5 eV.

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Legends for Figures

Figure 1. Ammonia (O), propionic acid (□), and carbonyl (Δ) yields as a function of N-acetylalanine concentration in oxygen-free solution at pH 7 under γ-radiolysis.

Figure 2. Effects of excitation scavengers on ammonia yields in oxygen-free, 2M N-acetylalanine solutions under γ radiolysis; benzene sulfonic acid (O), phenol (●), benzoic acid (◐), naphthalene sulfonic acid (◐).

Figure 3. Singlet (——) and triplet (---) energy levels of excitation scavengers used in the present study. The line (•••) represents the energy level of $RCONHCHR_2^*$.

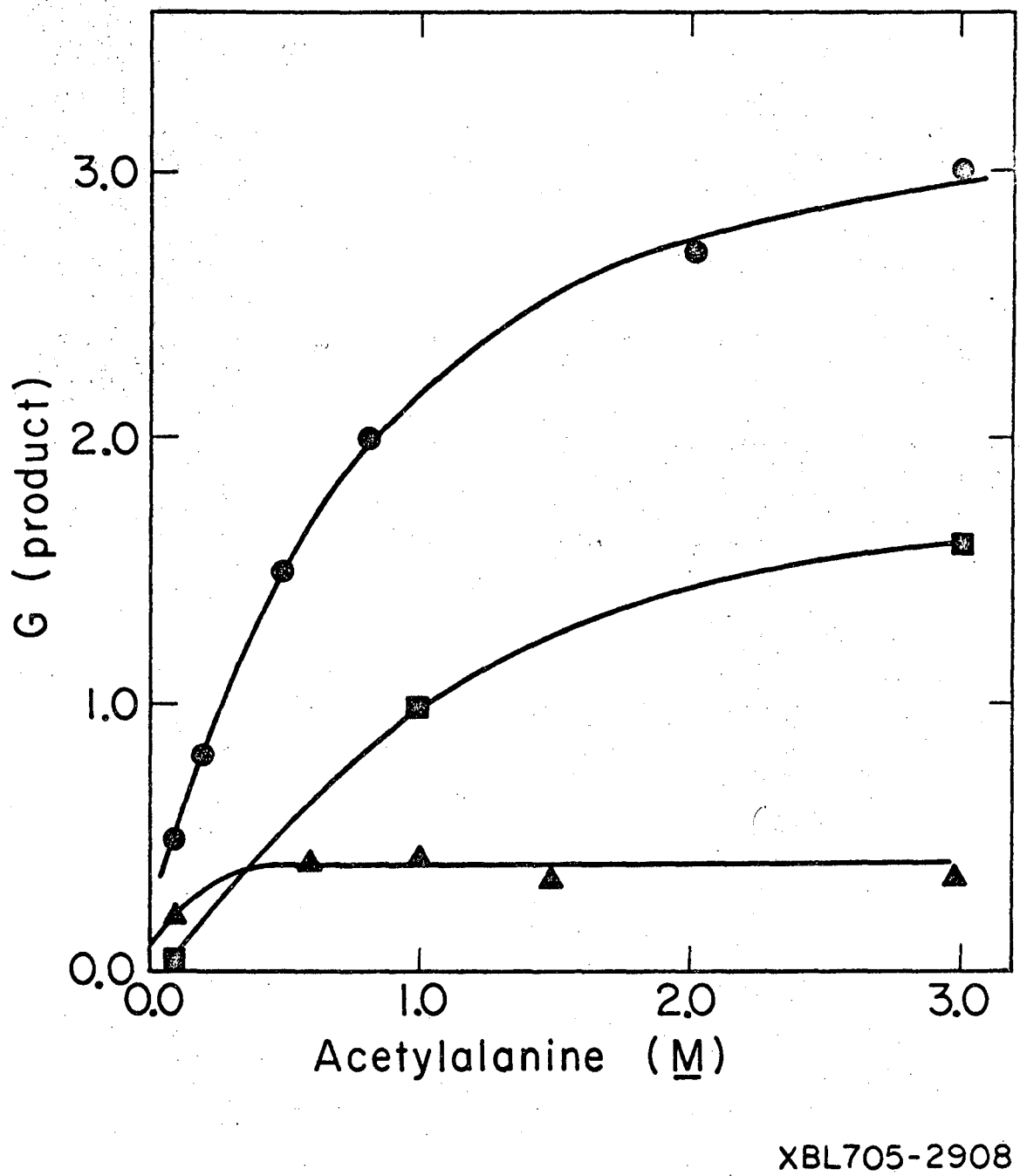

Fig. 1

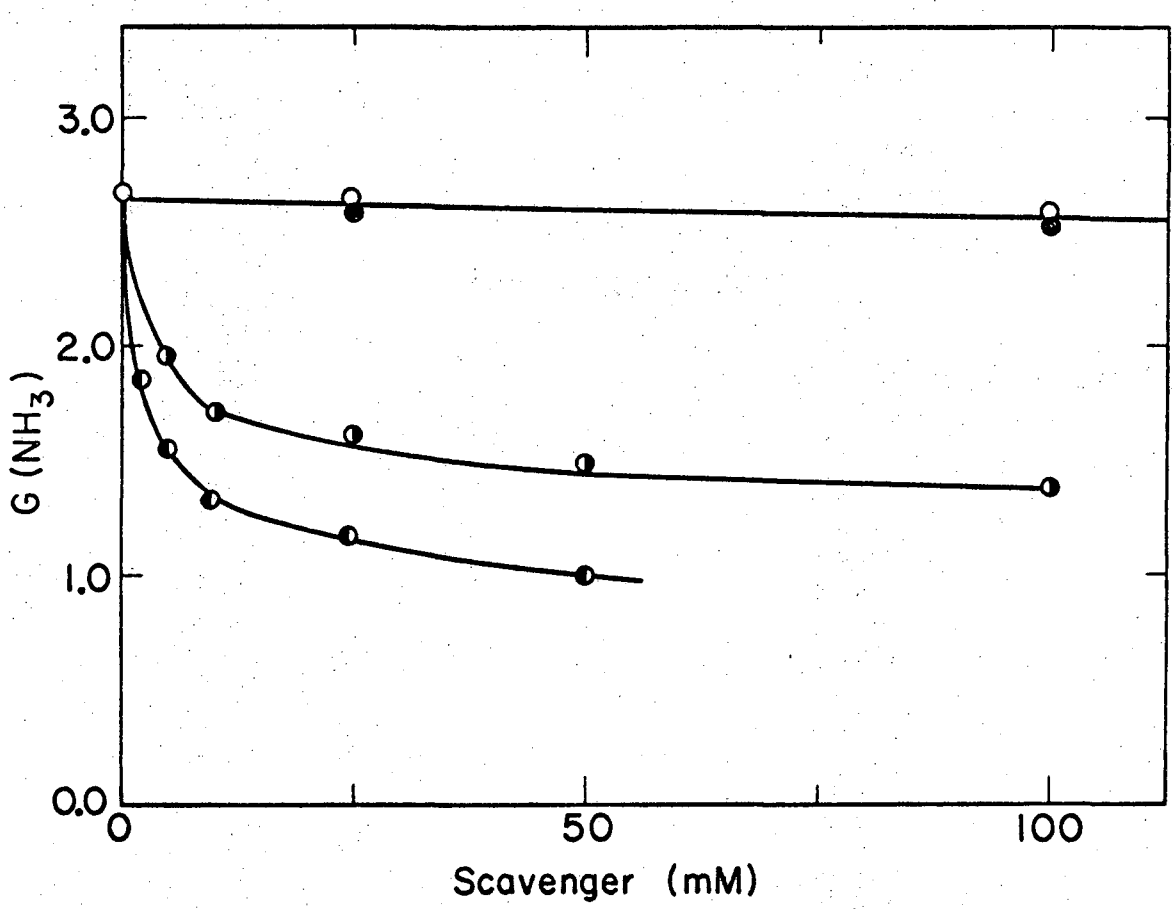

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Fig. 2

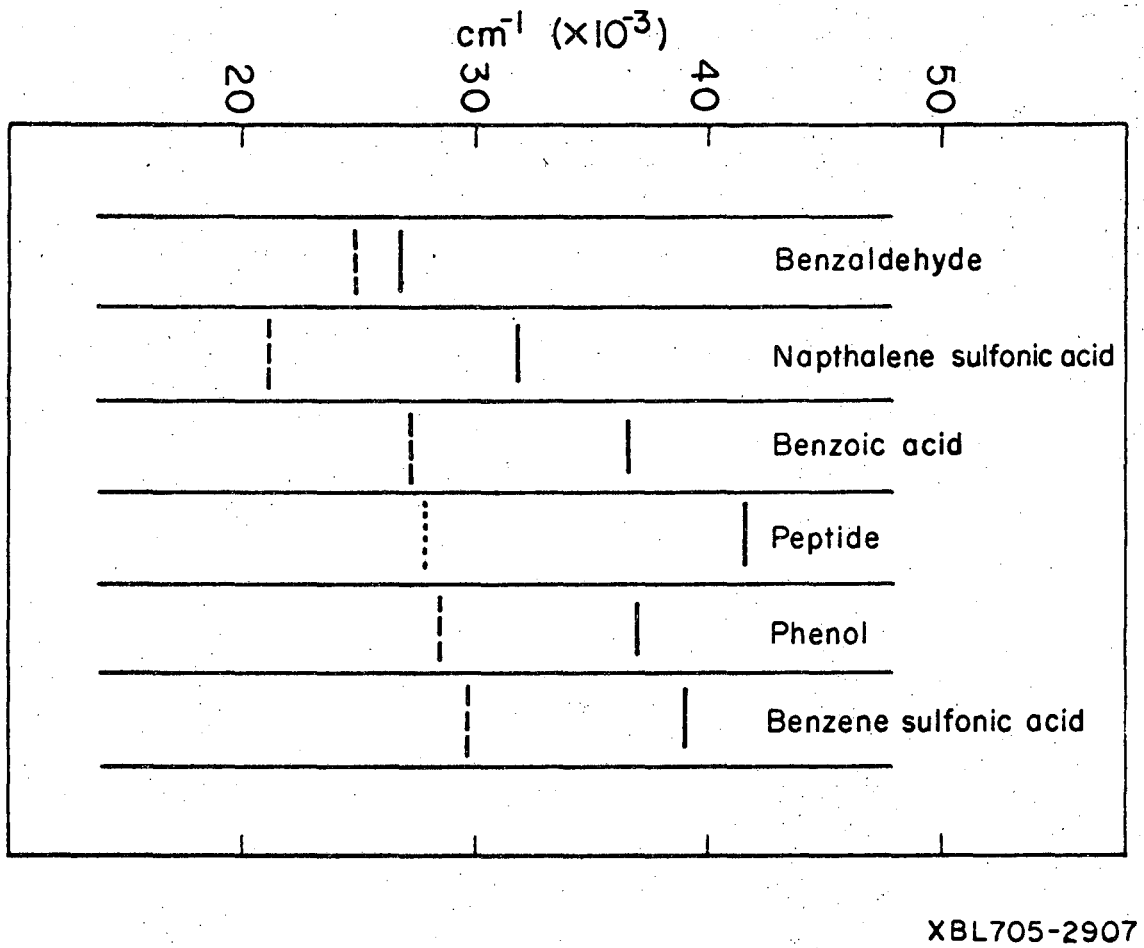
cm⁻¹ (×10⁻³)
20
30
40
50
Benzaldehyde
Napthalene sulfonic acid
Benzoic acid
Peptide
Phenol
Benzene sulfonic acid
XBL705-2907

Fig. 3

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