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A MARKED EFFECT OF CONFORMATION IN THE RADIOLYSIS OF POLY-α-L-GLUTAMIC ACID IN AQUEOUS SOLUTION

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A MARKED EFFECT OF CONFORMATION IN THE RADIOLYSIS OF POLY-α-L-GLUTAMIC ACID IN AQUEOUS SOLUTION

Harvey A. Sokol, Winifred Bennett-Corniea and Warren M. Garrison

October 1964

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ERRATA

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FROM: Technical Information Division

SUBJECT: UCRL-11733, "A Marked Effect of Conformation in the Radiolysis of Poly-α-L-Glutamic Acid in Aqueous Solution", Harvey A. Sokol, Winifred Bennett-Corniea and Warren M. Garrison, October 1964.

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7 Legend for Fig. 1: Effect of pH on the yield of ammonia (•), total α-keto acids (•) and α-Ketoglutaric acid (o) in the γ-radiolysis of 0.15 percent poly-α-L-glutamic acid. G = molecules/100 eV.

8 Fig. 1

A MARKED EFFECT OF CONFORMATION IN THE RADIOLYSIS OF POLY-α-L-GLUTAMIC ACID IN AQUEOUS SOLUTION

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Compounds containing the peptide bond undergo chemical degradation at the N-C linkage on irradiation in aqueous solution under oxygen. The schematics of the radiation-induced reactions are given by[1]

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

$$e^-_{aq} + O_2 + H_2O \rightleftharpoons HO_2 + OH^- \qquad (2)$$

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

$$O_2 + RCONH\dot{C}R_2 \longrightarrow RCONHC(\dot{O}_2)R_2 \qquad (4)$$

$$HO_2 + RCONHC(\dot{O}_2)R_2 \longrightarrow H_2O_2 + O_2 + RCON{=}CR_2 \qquad (5)$$

$$2RCONHC(\dot{O}_2)R_2 \longrightarrow H_2O_2 + O_2 + 2RCON{=}CR_2 \qquad (6)$$

$$2HO_2 \longrightarrow H_2O_2 + O_2 \qquad (7)$$

The dehydropeptides $RCON{=}CR_2$, as a class are readily hydrolyzed

$$RCON{=}CR_2 + 2H_2O \longrightarrow RCOOH + NH_3 + R_2CO \qquad (8)$$

in dilute mineral acid under the conditions conventionally employed to liberate ammonia from organic amides.[1,2] Hence, regardless of the relative rates of reactions 5 and 6, each $RCONH\dot{C}R_2$ radical yields one molecule of ammonia on hydrolysis of the irradiated system.[3] The over-all stoichiometry of steps 1 to 7 plus the hydrolysis steps is given by:

$$RCONHCHR_2 + O_2 + 2H_2O \longrightarrow RCOOH + NH_3 + R_2CO + H_2O_2 \quad (9)$$

We report here a study of the γ-ray induced oxidation of poly-α-L-glutamic acid (PGA) over the pH range 3.5 to 9. Dilute solutions of PGA (Pilot chemicals, Lot G-9, molecular weight, 140,000) were purified by prolonged dialysis, adjusted to the desired pH value through addition of NaOH or H_2SO_4, and irradiated under oxygen at one atmosphere in sealed pyrex tubes at a γ-ray dose-rate of 1.2×10^{18} eV/gm/min. The tubes were rotated at intervals to prevent depletion of oxygen in the liquid phase. A series of control runs established the time and speed of rotation required to insure the presence of excess oxygen under all conditions of pH. (PGA below pH 4 is metastable and precipitation occurs if the solution is agitated too vigorously). Following irradiation, the solutions were made 2 N in H_2SO_4, hydrolyzed under nitrogen for 3 hours at 100° C and assayed for ammonia[4] and carbonyl products.[1,5] Alpha ketoglutaric and pyruvic acids were identified as the major carbonyl products. Acetaldehyde and glyoxylic acid were detected in low yield. The validity of the analytical procedures was established through analysis of known mixtures of the four carbonyl products.

Product yields from 0.15 percent PGA solutions over the pH range 3.5 to 9 are summarized in Fig. 1. It is seen that the yield of ammonia and the combined yield of α-keto acids increase abruptly to their maximum values with

increasing pH over the narrow range pH ≃ 4.5 to pH ≃ 6. That this effect is not a result of incomplete scavenging of OH radicals at pH < 6 is shown by the fact that product yields at both pH 4 and pH 7 are independent of the PGA concentration from 0.15 percent down to at least .015 percent. Nor does it appear that the sharp break in the pH-yield curves is directly related to changes in hydrogen-ion concentration or degree of ionization of side-chain carboxyl groups, per se. This is shown by results obtained with N-acetylglutamic-α methyl ester, a radiation-chemical model for the single-residue segment of the PGA chain; ammonia and carbonyl yields from .05 M solutions of this low-molecular-weight peptide derivative of glutamic acid are essentially independent of pH over the entire range, pH 3 to 8, with $G(NH_3) \simeq G(>C=O) \simeq 2.2$.

The data of Fig. 1 show that G(pyruvic) increases sharply with $G(NH_3)$ with increasing pH from 4.5 to 6; whereas the yield of α-keto-glutaric is essentially pH independent in accord with the schematics of equations 5 and 6. The evidence is that pyruvic acid (plus ammonia) arises from a parallel reaction involving OH attack at a locus other than the peptide chain. After a detailed consideration of the possible chemical consequences of OH attack at each of the various C-H bonds of the glutamic acid residue, we conclude that pyruvic acid is produced as a consequence of OH attack at the C-H bond α to the side-chain carboxyl group. Consider the reactions:

$$OH + >CHCH_2CH_2COOH \longrightarrow H_2O + >CHCH_2\dot{C}HCOOH \qquad (10)$$

$$O_2 + >CHCH_2\dot{C}HCOOH \longrightarrow >CHCH_2CH(\dot{O}_2)COOH \qquad (11)$$

where $>CHCH_2CH_2COOH$ represents the side chain of the glutamic acid residue. Peroxy radicals of the type formed in reaction 11 are relatively long-lived and we suggest they are subsequently removed through the competing reactions

$$HO_2 + >CHCH_2CH(\dot{O}_2)COOH \longrightarrow O_2 + >CHCH_2CH(OOH)COOH \qquad (12)$$

and

$$2\ >CHCH_2CH(\dot{O}_2)COOH \longrightarrow >C{=}CH_2 + (COOH)_2 + >CHCH_2CH(OOH)COOH \qquad (13)$$

Degradation reactions akin to reaction 13 have been described by Russell[6] and by Durup et al.[7] It is seen that the product $>C{=}CH_2$ in the above nomenclature corresponds to the acrylic acid derivative $RCONHC(=CH_2)R$ (a tautomeric form of the dehydropeptide, $RCON{=}C(CH_3)R$) which on hydrolysis yields ammonia and pyruvic acid. And, we conclude from the results obtained with N-acetyl glutamic-α methyl ester that the relative rates of reactions 12 and 13 are essentially independent of the degree of ionization of the reacting species over the pH range 3 to 8.

Now, to explain the pronounced effect of pH on $G(NH_3)$ and G(pyruvic) from PGA we note first that a unique characteristic of the radiation chemistry of a macromolecular substance in aqueous solution is that each molecule undergoes reaction with a relatively large number of OH radicals even at the lowest practicable dosages. For example, with a 0.15 percent solution of PGA, a γ-ray dose of 3×10^{18} eV/gm produces but one OH per 100 glutamic acid residues but at the same time this corresponds to about 20 OH radicals per PGA molecule (140,000 MW). However, since PGA above pH 6 has the random coil configuration, the various segments of the macromolecule are free to interact both intermolecularly and intramolecularly and we find at pH > 6 no essential differences between the macromolecule and the low-molecular weight model from the standpoint of product yields. But, as the pH of the solution is decreased, PGA undergoes a coil $\rightarrow$ helix transition over the pH range 6 to 4.5 which as we have noted is the significant pH range of Fig. 1. With PGA in the helix form, the peroxy radicals are frozen in a fixed spatial arrangement and it is obvious

that the probability of reaction 13 is greatly reduced; hence reaction 12 is favored and as a result G(NH_3) and G(pyruvic) decrease as seen in Fig. 1.[9]

REFERENCES AND FOOTNOTES

(1) (a) W. M. Garrison, M. E. Jayko, W. Bennett-Corniea, Radiation Res. 16, 483 (1962). (b) W. M. Garrison and B. M. Weeks, Radiation Res. 17, 341 (1962).

(2) J. P. Greenstein and M. Winitz, Chemistry of the Amino Acids, John Wiley and Sons, Inc. New York, N. Y., 1961, p. 843.

(3) There is some evidence (ref. 1 b) that peroxy radicals of certain peptides give the amide directly without intermediate formation of the dehydro-peptide e. g. $HO_2 + RCONHC(\dot{O}_2)R_2 \xrightarrow{H_2O} RCONH_2 + R_2CO{+}O_2 + H_2O_2$.

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(7) M. Durup, J. Durup, F. Kuffer and M. Magat, Proc. 2nd Intern. Conf. Peaceful Uses Atomic Energy, Geneva, 1958, 29, 143.

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(9) This work was done under the auspices of the U. S. Atomic Energy Commission.

LEGEND FOR FIG. 1

Effect of pH on the yield of ammonia (o), total α-keto acids (o) and α-Ketoglutaric acid (o) in the γ-radiolysis of 0.15 percent poly-α-L-glutamic acid. G = molecules/100 eV.

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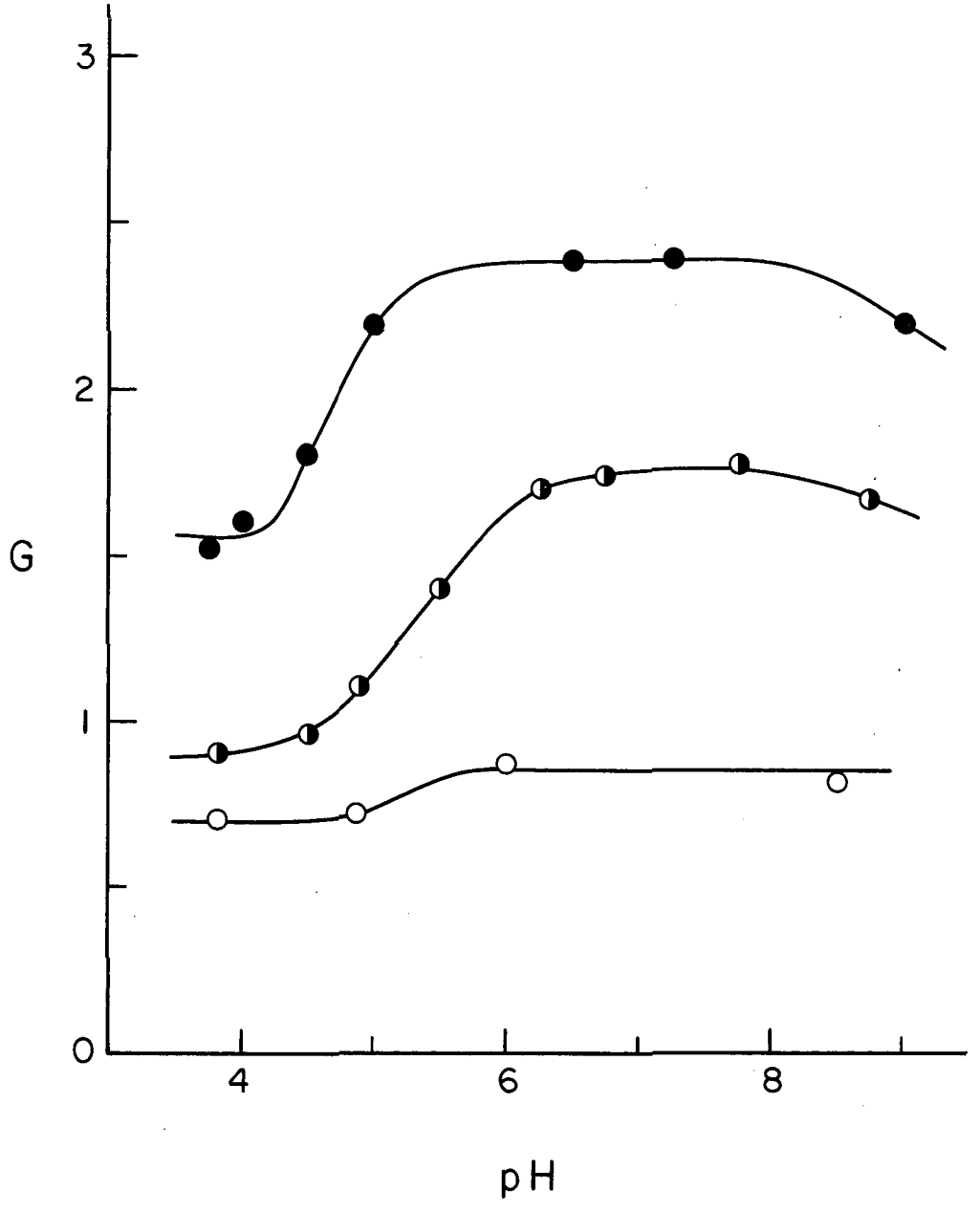

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Fig. 1

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