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PRODUCTION OF AMIDE GROUPS AND AMMONIA IN THE RADIOLYSIS OF AQUEOUS SOLUTIONS OF PROTEIN

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PRODUCTION OF AMIDE GROUPS AND AMMONIA IN THE RADIOLYSIS OF AQUEOUS SOLUTIONS OF PROTEIN

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Recent studies at this Laboratory have indicated[1,2] that a principal reaction in the radiolysis of aqueous solutions of protein under certain conditions involves cleavage of the peptide chain to form the carbonyl and amide functions as represented by the equation

$$-CO-NH-CH(R)- + O_2 + H_2O \longrightarrow -CONH_2 + RCO- + H_2O_2. \qquad (1)$$

Evidence for this type of radiation-induced oxidation has been derived largely from studies of carbonyl production in oxygenated solutions of pepsin and gelatin. The carbonyl function is found to be associated with high molecular weight (non-dialyzable) products which on conventional acid hydrolysis yield a series of α-keto acids. A correlation of these results in terms of a detailed mechanism for reaction (1) has been given elsewhere.[2]

Substantiating evidence for the reaction as written has now been obtained from studies of the amide content of reaction products formed by γ-ray-induced oxidation of aqueous gelatin. Commercially available lime-process gelatin was found to be particularly suitable for this study since most of the amide groups of the glutamine and asparagine residues of the parent collagen are removed through hydrolysis during the manufacturing process.[3] Unmodified proteins, in general, contain amide groups in sufficient number to mask reaction (1) at the lower radiation dosages necessarily invoked in studies of initial reaction products.

Analyses were made for "free" ammonia and for amide groups, both before and after irradiation. The stock solutions (3.5 per cent gelatin, purified

calfskin, Eastman Lot 60-9630) had been previously dialyzed to reduce the ammonia content to a minimum value. Analytical procedures were, in brief, as follows: An appropriate aliquot of the solution was made alkaline to phenolphthalein and distilled at room temperature in vacuo into a receiver which contained 1 ml of 0.1 N sulfuric acid at the temperature of liquid nitrogen. The distillate was isolated, thawed, and then assayed for ammonia by means of the Nessler reaction. A second aliquot of each solution was made 1 N in hydrochloric acid and heated for one hour at 95°C to liberate ammonia from amide linkages.[4] Subsequent treatment was then as described for ammonia. A series of control runs showed that the liberation of amide-ammonia was essentially quantitative under the mild hydrolysis conditions employed. Prolonged hydrolysis leads to the gradual break-down of various labile amino acids with the formation of what in this study would be "artifact" ammonia.[5,6] An increase of 25 per cent in the number of amide groups in an irradiated solution as compared to the corresponding control solution could easily be observed with the method outlined above. Data shown in Table I were obtained on solutions that had been irradiated until the amide and ammonia content had reached values that were at least three times those of the control.

A 2000 curie Co^{60} γ-ray source was used in the irradiations. Solutions (10 ml) were exposed under oxygen (5 ml) at one atmosphere in sealed pyrex tubes. Contents were shaken at intervals to prevent depletion of oxygen in the solutions. The dose rate was 2.5×10^{18} ev/ml/min.

The data of Table I indicate that the formation of amide groups ($G \simeq 1$) corresponds to a principal reaction in the radiolysis of aqueous solutions of gelatin. Further, the G value for this product-group is very close to that obtained for total carbonyl formation under equivalent irradiation

conditions.[2] The simplest explanation for these results would seem to involve the postulated reaction (1). Ammonia formation ($G \sim 0.3$) may be attributed to a radiation-induced oxidation of terminal and/or side-chain amino groups.[7]

This work was performed under the auspices of the United States Atomic Energy Commission.

Table I

Production of amide groups and ammonia in the γ-ray radiolysis of aqueous gelatin solutions

	Yield (G)*	
	Series 1	Series 2
Amide Groups	1.0 ± .1	0.9 ± .1
Ammonia	0.25 ± .05	0.30 ± .05

* Yield per 100 ev absorbed energy.

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