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ON THE RADIATION-INDUCED REDUCTION OF AMIDE AND PEPTIDE FUNCTIONS IN AQUO-ORGANIC SYSTEMS

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John Holian and Warren M. Garrison

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John Holian and Warren M. Garrison

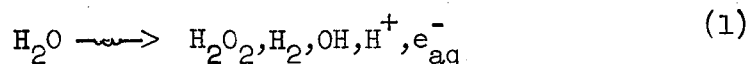
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ON THE RADIATION-INDUCED REDUCTION OF AMIDE AND  
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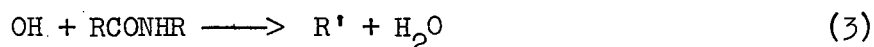
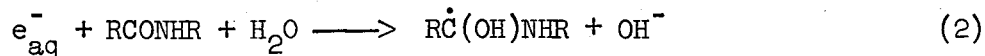
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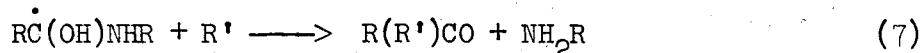
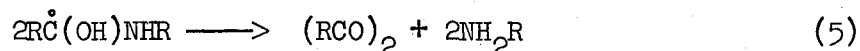
In the  $\gamma$ -radiolysis of neutral, oxygen-free solutions of peptides such as N-acetylglycine and N-acetyl alanine, the labile products of the radiation-induced step<sup>2,3</sup>



are removed through reactions of the type<sup>4,5</sup>



In subsequent chemistry, the radical combination reactions which lead to net reductive cleavage of the amide linkage



occur in relatively low yield. We find, for example, that the combined yield of carbonyl products  $(RCO)_2$ ,  $RCHO$ ,  $R(R')CO$  in neutral solutions of N-acetyl-glycine and N-acetyl alanine, corresponds to  $G(> CO) \leq 0.2$ .<sup>4b</sup> The indirect evidence is that a reconstitution reaction with the stoichiometry



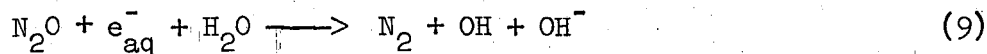
represents an important path for removal of the radical species derived from  $RCONHCH(R)COO^-$  through reactions 2-4.

By formulating reaction 8 as a major termination reaction in the radiolysis of these systems we have assumed that the  $C = O$  linkage of the carboxylate group of the acylamino acid does not compete effectively as a trap for  $e_{aq}^-$ . This would seem to be a valid assumption since the rate constants for reaction of  $e_{aq}^-$  with acetyl-glycine and acetyl-alanine ( $RCONHCHRCOO^-$ ) are of the order,<sup>4a</sup>  $k \sim 10^8 M^{-1} sec^{-1}$  whereas for reactions of  $e_{aq}^-$  with the simple fatty acids ( $RCOO^-$ ) the rates are low,<sup>6</sup>  $k < 10^6 M^{-1} sec^{-1}$ .

However, the most appropriate model compounds for study of reactions 2 to 8 are, of course, those in which the  $C = O$  group of the amide function represents the only locus of unsaturation.

Accordingly, we have investigated the analogues of reactions 2 to 8 in oxygen-free solutions of acetamide and of N-ethylacetamide under  $\gamma$ -radiolysis. And, because of the relative simplicity of the chemistry of the aqueous acetamide system we have been able on the basis of stoichiometric considerations to wholly substantiate the validity of the proposed reaction scheme given in eqs. 1 to 8. We report here a brief description of the experimental findings.<sup>7</sup>

To establish the locus of OH attack on acetamide and to eliminate any contribution of the back-reaction 8, we make use of the fact that dissolved  $N_2O$  converts  $e_{aq}^-$  to OH via <sup>8,9</sup>



where  $k_9 = 5.6 \times 10^9$ .

The data of Table I, column 1, show that the OH radicals formed via reactions 1 and 9 react preferentially at the methyl group of acetamide to yield the radicals  $\dot{C}H_2CONH_2$  which subsequently dimerize to give  $G(\text{succinamide}) = 2.6 \simeq [G_{OH} + G_{e_{aq}^-} + G_H]/2$ . That the H atom also reacts with acetamide to yield  $\dot{C}H_2CONH_2$  is shown by the data obtained with 0.25 M acetamide at pH 1 (Table I, column 2); In acidic solution  $e_{aq}^-$  is converted to H via



where  $k_{10} = 2.3 \times 10^{10}$ . Hence at pH 1 we find  $G(H_2) = 3.8 \simeq G_H + G_{e_{aq}^-} + G_{H_2}$ ;  $G(\text{succinamide}) = 2.5 \simeq [G_{OH} + G_{e_{aq}^-} + G_H]/2$ . The yield of carbonyl products is essentially zero in solutions containing the electron scavengers  $H^+$ ,  $N_2O$ .

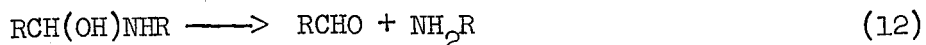
If now we examine product yields in evacuated, 0.25 M acetamide solutions at pH 7 we find as shown in Table 1, column 3 that succinamide is produced in quite low yield,  $G(\text{succinamide}) \simeq 0.3$ . The combined yield of carbonyl products is also low with  $G(>CO) < 0.2$ . Detailed analytical probes of this relatively simple system have failed to reveal any other organic products in significant yield.

Since we know from the rate data that both  $e_{aq}^-$  and OH are quantitatively scavenged by acetamide in 0.25 M solution at pH7, we are led to conclude that radical termination in this system occurs almost exclusively through the reconstitution reaction 8.

Direct substantiating evidence for the occurrence of such reaction in neutral solutions of amides and peptides has also been obtained. We find that certain labile organic compounds, notably the thiols, RSH, are effective at low concentrations in blocking the back-reaction 8 by virtue of the H-atom transfer reaction



which leads in turn to the cleavage of the amide (peptide) linkage



As indicated in Table II, acetaldehyde appears as the major product with  $G(CH_3CHO) \simeq 2.5$  in solutions of acetamide and N-ethylacetamide containing the thiol, cysteine.

It is to be noted that the thiols, because of their marked reactivity, are ordinarily found to act as protective agents in the radiolysis of aqueous systems.<sup>10</sup> Of interest from both the chemical and biological standpoint is the present finding that RSH at low concentrations induces a very striking enhancement in the radiolytic lability of the amide (peptide) linkage.

A detailed report of this work is in preparation.



## REFERENCES AND FOOTNOTES

1. This work was done under the auspices of the United States Atomic Energy Commission.
2. a) A. O. Allen, Radiation Res. Suppl. 4, 54 (1964); b) E. J. Hart and R. L. Platzman, Mech. Radiobiology 1, 93 (1961); c) C. S. Hochanadel and R. Casey, Radiation Res. 25, 198 (1965).
3. Recent measurements (ref. 2c) of the 100 eV yields for reaction 1 give  $G_{OH} = 2.59$ ,  $G_{e^-} = 2.58$ ,  $G_H = 0.55$ ,  $G_{H_2} = .45$ ,  $G_{H_2O_2} = 0.72$ .
4. a) R. L. Willix and W. M. Garrison, Radiation Res. 32, 452 (1967); b) M. A. J. Rodgers, H. A. Sokol and W. M. Garrison, J. Am. Chem. Soc. 90, 795 (1968); c) W. M. Garrison, Current Topics in Radiation Research, Vol. IV, 1968; d) H. L. Atkins, W. Bennett-Gorneia and W. M. Garrison, J. Phys. Chem. 71, 772 (1967).
5. a) For purposes of simplicity, we use the general formulation, RCONHR, to represent both the primary amide and the monosubstituted primary amide (peptide); b) In the radiolysis of N-acetylglycine and N-acetyl alanine, OH and H attack occur at C-H linkage of the main chain, i.e.,  $R' = RCONH\dot{C}(R)COOH$  (ref. 4c).
6. For a recent compilation of rate data see M. Anbar and P. Neta, J. Appl. Radiation Isotopes 17, 493 (1967).
7. Experimental procedures and analytical methods used in this work have been described in ref. 4.
8. F. S. Dainton and D. B. Peterson, Proc. Roy. Soc. (London) A267, 443 (1962).

9. Rate constants,  $k_2$ , for reaction of  $e_{aq}^-$  with acetamide and N-ethylacetamide are  $1.7 \times 10^7$  and  $2 \times 10^7 M^{-1} sec^{-1}$ , respectively (ref. 4a,b).
10. For example see, A. O. Allen, Radiation Chemistry of Water and Aqueous Solutions (Van Nostrand Pub. Co., New York, 1961).

Table I.

Product yields in the  $\gamma$ -radiolysis of oxygen-free solutions of acetamide.<sup>a</sup>

Product	G Value		
	pH7 (+.02M N <sub>2</sub> O)	pH1	pH7
Succinamide	2.6	2.5	0.3
Acetaldehyde	{ < .05	{ < .05	0.1
Other Carbonyls <sup>b</sup>			~ .03
Hydrogen	<sup>c</sup>	3.8	0.9

a. 0.25 M

b. Acetone plus biacetyl

c. Not measured

Table II.

Effect of cysteine (RSH) on the  $\gamma$ -ray induced reduction of acetamide and N-ethylacetamide in oxygen-free solution.

Amide Solution ( <u>M</u> pH7) <sup>a</sup>	(RSH), <u>M</u>	G(CH <sub>3</sub> CHO)
acetamide	none	~ 0.1
acetamide	$4 \times 10^{-4}$	2.4 <sup>b</sup>
N-ethylacetamide	none	< .05
N-ethylacetamide	$4 \times 10^{-4}$	2.8 <sup>c</sup>

- a. Since the rate constant for reaction of cysteine with  $e_{aq}^-$  corresponds to  $k = 2 \times 10^{10} \text{ M}^{-1} \text{ sec}^{-1}$  (ref. 6) it was necessary in this series of experiments to increase the amide concentration to 1M to insure the preferential scavenging of  $e_{aq}^-$  by RCONHCHR. Concentrations of RSH much below  $4 \times 10^{-4} \text{ M}$  are experimentally impracticable because of excessive depletion of the mercaptan during radiolysis.
- b. This yield is dose dependent and the value 2.4 represents the extrapolated yield at zero dose.
- c. At dosages below  $2.5 \times 10^{18} \text{ eV/gm}$ .

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