Lawrence Berkeley National Laboratory
Recent Work

Title

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

Permalink

https://escholarship.org/uc/item/721626qc

Authors

Holian, John
Garrison, Warren M.

Publication Date

1968-08-01

eScholarship.org Powered by the California Digital Library
University of California

UCRL-18428

eg. 2

RECEIVED
LAWRENCE
RADIATION LABORATORY
OCT 3 1968
LIBRARY AND
DOCUMENTS SECTION

University of California

Ernest O. Lawrence Radiation Laboratory

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

John Holian and Warren M. Garrison

August 1968

TWO-WEEK LOAN COPY

This is a Library Circulating Copy which may be borrowed for two weeks. For a personal retention copy, call Tech. Info. Division, Ext. 5545

Berkeley, California

UCRL-18428
Cy. 2

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

Submitted to: Journal of Physical Chemistry

UCRL 18428
Preprint

UNIVERSITY OF CALIFORNIA

Lawrence Radiation Laboratory
Berkeley, California

AEC Contract No. W 7405 eng 48

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

John Holian and Warren M. Garrison

August 1968

ON THE RADIATION-INDUCED REDUCTION OF AMIDE AND PEPTIDE FUNCTIONS IN AQUO-ORGANIC SYSTEMS[1]

John Holian and Warren M. Garrison

Lawrence Radiation Laboratory
University of California
Berkeley, California 94720

In the γ-radiolysis of neutral, oxygen-free solutions of peptides such as N-acetylglycine and N-acetyl alanine, the labile products of the radiation-induced step[2,3]

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

are removed through reactions of the type[4,5]

$$e^-_{aq} + RCONHR + H_2O \longrightarrow R\dot{C}(OH)NHR + OH^- \qquad (2)$$

$$OH + RCONHR \longrightarrow R' + H_2O \qquad (3)$$

$$H + RCONHR \longrightarrow R' + H_2 \qquad (4)$$

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

$$2R\dot{C}(OH)NHR \longrightarrow (RCO)_2 + 2NH_2R \qquad (5)$$

$$2R\dot{C}(OH)NHR \longrightarrow RCHO + NH_2R + RCONHR \qquad (6)$$

$$R\dot{C}(OH)NHR + R' \longrightarrow R(R')CO + NH_2R \qquad (7)$$

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-acetylglycine and N-acetyl alanine, corresponds to $G(> CO) \leq 0.2$.[4b] The indirect evidence is that a reconstitution reaction with the stoichiometry

$$R\dot{C}(OH)NHR + R' \longrightarrow 2RCONHR \qquad (8)$$

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 acetylglycine and acetylalamine ($RCONHCHRCOO^-$) are of the order,[4a] $k \sim 10^8 M^{-1}sec^{-1}$ whereas for reactions of e^-_{aq} with the simple fatty acids ($RCOO^-$) the rates are low,[6] $k < 10^6 M^{-1}sec^{-1}$.

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 γ-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.[7]

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[8,9]

$$N_2O + e^-_{aq} + H_2O \longrightarrow N_2 + OH + OH^- \qquad (9)$$

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 subsequentially dimerize to give G(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

$$e^-_{aq} + H^+ \longrightarrow H \qquad (10)$$

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 pH7 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

$$R\dot{C}(OH)NHR + RSH \longrightarrow RCH(OH)NHR + R\dot{S} \qquad (11)$$

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

$$RCH(OH)NHR \longrightarrow RCHO + NH_2R \qquad (12)$$

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.[10] 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-Corneia 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' = RCONHĊ(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 1.7×10^7 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 γ-radiolysis of oxygen-free solutions of acetamide.[a]

Product	G Value		
	pH7 (+.02M N_2O)	pH1	pH7
Succinamide	2.6	2.5	0.3
Acetaldehyde	< .05	< .05	0.1
Other Carbonyls[b]			~ .03
Hydrogen	[c]	3.8	0.9

a. 0.25 M

b. Acetone plus biacetyl

c. Not measured

Table II.

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

Amide Solution (M pH7)[a]	(RSH), M	G(CH_3CHO)
acetamide	none	~ 0.1
acetamide	4×10^{-4}	2.4[b]
N-ethylacetamide	none	< .05
N-ethylacetamide	4×10^{-4}	2.8[c]

a. Since the rate constant for reaction of cysteine with e^-_{aq} corresponds to $k = 2 \times 10^{10} M^{-1} sec^{-1}$ (ref. 6) it was necessary in this series of experiments to increase the amide concentration to 1M to insure the preferential scavening of e^-_{aq} by RCONHCHR. Concentrations of RSH much below 4×10^{-4} 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 2.5×10^{18} eV/gm.

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or

B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.