Lawrence Berkeley National Laboratory
Recent Work

Title
RADIATION-CHEMICAL EVIDENCE FOR EXCITED ELECTRONIC STATES OP THE PEPTIDE BOND

Permalink
https://escholarship.org/uc/item/0357p929

Authors
Garrison, Warren M.
Jayko, Michael E.
Bennett-Corniea, Winifred.

Publication Date
1964-04-16

University of California

Ernest O. Lawrence Radiation Laboratory

RADIATION-CHEMICAL EVIDENCE FOR EXCITED ELECTRONIC STATES
OF THE PEPTIDE BOND

Berkeley, California

DISCLAIMER

UNIVERSITY OF CALIFORNIA

RADIATION-CHEMICAL EVIDENCE FOR EXCITED ELECTRONIC STATES OF THE PEPTIDE BOND

Warren M. Garrison, Michael E. Jayko, and Winifred Bennett-Corniea

April 16, 1964

RADIATION-CHEMICAL EVIDENCE FOR EXCITED ELECTRONIC STATES
OF THE PEPTIDE BOND

Warren M. Garrison, Michael E. Jayko, and Winifred Bennett-Corniea

Lawrence Radiation Laboratory
University of California
Berkeley, California

ABSTRACT

Gamma radiolysis of polypeptides under oxygen leads to chemical

degradation of the peptide chain with the remarkably high oxygen consumption

of about one molecule per 2 eV absorbed energy. The chemical evidence, both

qualitative and quantitative is consistent with a reaction mechanism in which

excited states of the polypeptide aggregate undergo chemical quenching by

molecular oxygen.

Excited states involving delocalized π electrons of the extended hydrogen bond system (CO--HN)n of polypeptides have been proposed as intermediates in the processes of energy transfer, photosynthesis, vision and carcinogenesis (1-6). Measurements of semiconduction activation energies indicate that the excited levels are 1.5 to 3 eV above the ground state in dry proteins (7). Mason (6) treats π-electron delocalization in polypeptides in terms of a triplet-state excitation which is assumed to involve enolization of the peptide bond, i.e. ~CO(OH)=N~ or ~C(R)=C(OH)-NH~ or both. Mason also supposes that such states represent essential intermediates in the radiation-induced denaturation of protein. Now, low-lying excited states of the peptide bond system as envisaged here would not be expected to lead to net chemical change in the radiolysis of peptides in vacuo. There is, however, the possibility that these excited states may be chemically reactive toward molecular oxygen. Yet, the available evidence obtained from the work of Alexander and Hamilton (8) and Bowes and Moss (9) indicates that the radiation-induced destruction of amino acids in solid proteins is not significantly greater under oxygen than it is in vacuo. But, in the one study (8) the irradiations were made at the very high dose rate of ~5 x 10^{21} eV/gm/min and in the other (9) the solids were irradiated in a compacted form. In neither case would we expect the oxygen effect to be more than minimal. We have, therefore, re-examined this question by irradiating a polypeptide, gelatin, and a polyamino acid, poly-D,L-alanine (10) as highly dispersed fluffs (average bulk density ~.05gm/cc) in vacuo and in an oxygen atmosphere under γ-rays at the relatively low dose-rate of 1 x 10^{16} eV/gm/min.

The very striking result shown in Table I is the remarkably high G values for oxygen uptake and for oxidative degradation of both gelatin and

poly-D,L-alanine. Chemical analysis of the irradiated peptides following

a 3 hour hydrolysis in 2$\underline{N}$ hydrochloric acid established ammonia and α-ketoacid(s)

as principal degradation products. The quantitative procedures were those

developed in our previous studies of the radiolytic oxidation of polypeptides

in aqueous solution (11). Oxygen uptake and carbon dioxide production were

followed mass spectrometrically. The observed $G(-O_2)$ values correspond to

an energy requirement of only 1.5 and 2.0 eV per molecule of oxygen removed

by gelatin and poly-D,L-alanine respectively. These energies are much lower

than can be explained in terms of homolytic or heterolytic dissociations of
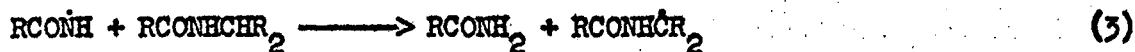
specific groups or bonds.

Evidence against a chain reaction as the cause of the high $G(-O_2)$

values is as follows. We note first that in the radiolysis of polypeptides

in aqueous solution (11) the radical species $RCON\overset{\bullet}{H}CR_2$ are formed by OH

attack and are degraded in the presence of oxygen via.

$$RCON\overset{\bullet}{H}CR_2 + O_2 \longrightarrow RCONHC(\overset{\bullet}{O}_2)R_2 \xrightarrow{H_2O} RCONH_2 + R_2CO + HO_2 \qquad (1)$$
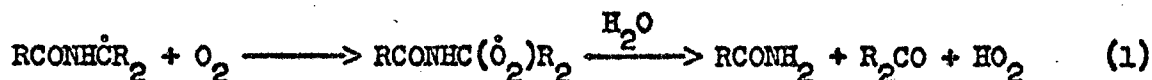
The ammonia yield from oxygenated peptide solutions approaches but never

exceeds the primary yield of OH radicals derived from the radiation

decomposition of water. Neither $RCONHC(\overset{\bullet}{O}_2)R_2$ or HO_2 radicals abstract H

from the labile C-H position α to the peptide bond and no chain oxidation

is observed in aqueous systems. Now, the radicals $RCON\overset{\bullet}{H}CR_2$ are also

produced in the radiolysis of solid peptides $\underline{in\ vacuo}$. (12, 13). The

formation of amide ammonia and carbonyl products reported in part A
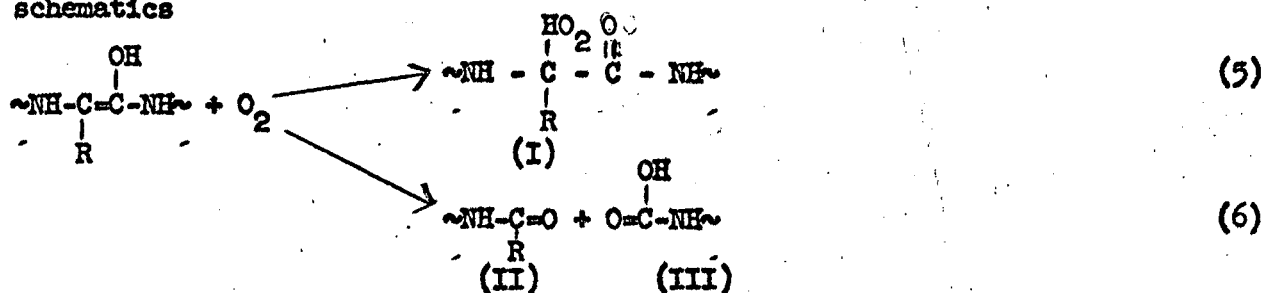
Table I involves the radiation-induced step 2.

$$RCONHCHR_2 \xrightarrow{\sim\!\!\|\!\|\!\!\sim} RCON\dot{H} + \dot{C}HR_2 \tag{2}$$

followed by

$$RCON\dot{H} + RCONHCHR_2 \longrightarrow RCONH_2 + RCONH\dot{C}R_2 \tag{3}$$

$$\dot{C}HR_2 + RCONHCHR_2 \longrightarrow CH_2R_2 + RCONH\dot{C}R_2 \tag{4}$$

where $RCOHN\dot{C}R_2$ represents the long-lived radicals observed by ESR

methods (14) in irradiated peptides at room temperature. Exposure of

the irradiated peptides to air during the dissolution process results,

as in the radiolysis of the oxygenated solutions, in the degradation of

$RCONH\dot{C}R_2$ radicals via reaction 1 to give the product yields shown in

part A Table I. These product yields are in essential accord with the

ESR measurements (14) of $RCONH\dot{C}R_2$ yields and here again we find no evidence

for chain oxidation. Since neither $RCONHC(\dot{O}_2)R_2$ nor HO_2 abstract H from

the labile C-H position we conclude that the high G values for oxygen

consumption shown in part B Table I do not arise from a chain oxidation

under the conditions of the present study.

Both the qualitative and quantitative evidence is in accord with

the idea that the greatly enhanced degradation yields obtained in the

radiolysis of highly dispersed polypeptide under oxygen is related to the

mobility and to the chemical reactivity towards oxygen of low-lying

excited states which are chemically undetected _in vacuo_. We represent the

oxidation of the excited state of the peptide bond system in terms of the

schematics

$$\sim\!NH\!-\!\underset{\underset{R}{|}}{C}\!=\!C\!-\!NH\!\sim + O_2 \longrightarrow \sim\!NH - \underset{\underset{R}{|}}{\overset{HO_2}{\underset{|}{C}}} - \overset{O}{\overset{\|}{C}} - NH\!\sim \tag{5}$$

$$(I)$$

$$\longrightarrow \sim\!NH\!-\!\underset{R}{\overset{|}{C}}\!=\!O + O\!=\!\overset{OH}{\overset{|}{C}}\!-\!NH\!\sim \tag{6}$$

$$(II) \qquad\qquad (III)$$

where on hydrolysis the ammonia is derived from I and II, α-ketoacid from
I, and carbon dioxide from III. The magnitude of the observed G values for
reactions 5, 6 prompted us also to give particular attention to the quantitative
basis of the present work. Dose was determined by extrapolation on the basis
of the ratio of the electron densities of the solids and the Fricke dosi-
meter (15). The validity of the extrapolation for the low-density solids
and the geometries used here was extablished experimentally in a series of
control runs in which the density and the volume of the irradiated samples
was varied from ~.025 gm/cc to 0.6 gm/cc and from 1cc to 80 cc respectively.
The possibility that not all of the observed chemical change arises as a
result of the direct action of the radiation on the polypeptides was examined
in some detail. The fact that the degradation yields in oxygen are indepen-
dent of pressure from 650mm down to 20mm indicates that energy absorption by
oxygen is not a major contributing factor. At 20mm pressure the oxygen in
the gas phase plus that absorbed on the solid peptide represents less than
1% of the mass of the total sample (16). The possibility that part of the
energy absorbed by the pyrex cell is transferred to the peptide through
excited states of oxygen is negated by the observed pressure independence
and by the observation that addition of a glass-fibre plug above the peptide
sample does not result in any change in the degradation yields. There is
also the question as to whether or not the amide and carbonyl yields given
in Table I includes some contributions from "dark-reactions" induced in the
hydrolysis step. Both peroxides and carbonyl compounds for example, are
known to degrade amino acids via the Strecker reaction with formation of
ammonia under certain conditions (17). We found, however, in a series of
controls that added hydrogen peroxide, organic peroxides and carbonyl

compounds in amounts corresponding to G~40 do not lead to oxidation of the peptide chain under the acid hydrolysis conditions employed in this study. We also found that treatment of the irradiated polypeptide with 0.1N Na_2SO_3 to destroy peroxides prior to hydrolysis does not lead to any diminution in product yields. These findings are in accord with earlier oberservations (11) on the negative rôle of dark-reactions in the chemical determination of radiation-induced oxidation in aqueous peptide systems (18).

Warren M. Garrison

Michael E. Jayko

Winifred Bennett-Corniea

University of California

Lawrence Radiation Laboratory

Berkeley, California

Table 1. Product yields in the γ-radiolysis of gelatin and poly-D,L-alanine in the solid state. A, irradiated _in vacuo_ and then exposed to oxygen prior to chemical manipulation. B, irradiated in oxygen at a pressure of 50mmHg. Dose = 1.44×10^{20} eV/gm. G = molecules per 100 eV absorbed energy.

	$G(>C = O)$	$G(NH_3)$	$G(CO_2)$	$G(-O_2)$
A. Evacuated				
gelatin	2.3 ± .3	8.1 ± .5	.63 ± .03	——
poly-D,L-alanine	4.8 ± .3	5.6 ± .3	.41 ± .01	——
B. Oxygen atmosphere				
gelatin	17.9 ± .9	42.1 ± 1.8	15.7 ± 3.0	72.7 ± .5
poly-D,L-alanine	24.3 ± .7	30.9 ± 1.7	33.8 ± .5	52.3 ± .9

Table 2. Effect of oxygen pressure on product yields in the γ-radiolysis of gelatin. Dose $= 1.44 \times 10^{20}$ eV/gm. G $=$ molecules per 100 eV absorbed energy.

Pressure (mmHg)	$G(> C = 0)$	$G(NH_3)$	$G(-O_2)$
20	$17.9 \pm .9$	42.1 ± 1.8	70.8
50	$18.8 \pm .9$	42.5 ± 2.0	$72.4 \pm .5$
600	16.4 ± 1.0	40.0 ± 2.5	——

REFERENCES AND NOTES

1. A. Szent-Györgi, Nature 148, 157 (1941).

2. M. G. Evans and J. Gergely, Biochim. Biophys. Acta 3, 188 (1949).

3. N. Riehl, Naturwissenschaften 43, 145 (1956).

4. W. Arnold and H. K. Sherwood, Proc. Natl. Acad. Sci. U. S. 43, 105 (1957).

5. B. Rosenberg, R. A. Orlando and J. M. Orlando, Arch. Biochem. Biophys. 93, 395 (1961).

6. R. Mason, Faraday Soc. Discussions 7, 129 (1959).

7. B. Rosenberg, J. Chem. Phys. 36, 816 (1962).

8. P. Alexander and L. D. G. Hamilton, Radiation Res. 13, 214 (1960).

9. J. H. Bowes and J. A. Moss, Radiation Res. 16, 211 (1962).

10. One percent solutions of gelatin (Eastman, lime processed) and poly-D, L-alanine (Yeda, M. W. 2000) in water and glacial formic acid respectively were dialyzed against distilled water, quick-frozen in liquid nitrogen and vacuum-dried at about -5°C.

11. W. M. Garrison, M. E. Jayko and W. Bennett, Radiation Res. 16, 483 (1962).

12. W. M. Garrison and B. M. Weeks, Radiation Res. 17, 341 (1962).

13. R. C. Drew and W. Gordy, Radiation Res. 18, 552 (1963).

14. T. Henriksen, T. Sanner and A. Pihl, Radiation Res. 18, 147 (1963).

15. C. J. Hockanadel and J. A. Ghormley, J. Chem. Phys. 21, 880 (1953).

16. S. W. Benson and D. A. Ellis, J. Am. Chem. Soc. 70, 3563 (1948).

17. A. Schonberg and R. Moubacher, Chem. Rev. 50, 261 (1952).

18. This work was done under the auspices of the U. S. Atomic Energy Commission.