Radiation Damage to Polypeptides and Proteins in the Solid State, II*
Radiolysis of Poly-L-Glutamic Acid

T. Söylemez, W. Baumeister

Institut für Biophysik und Elektronenmikroskopie der
Universität Düsseldorf, Moorenstraße 5,
D-4000 Düsseldorf, Bundesrepublik Deutschland

and L. M. Herbertz

Diabetes-Forschungsinstitut (Abteilung für Biochemie) der
Universität Düsseldorf, D-4000 Düsseldorf 1,
Bundesrepublik Deutschland

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For the transformation of glutamic acid into α-aminobutyric acid upon irradiation a decarboxylation mechanism involving the formation of CO_2 has been proposed previously. Here we present further experimental evidence in favour of this mechanism. Additionally the formation of CO as a decarboxylation product has been detected; a radical anion mechanism for its formation is proposed.

Introduction

The interaction of energetic electrons with the specimen entails chemical changes and may seriously deteriorate its structural organization; this imposes a fundamental problem to electron microscopy of organic materials. In a series of investigations [1 – 4] we make an attempt to characterize the kind and amount of radiation damage we have to encounter when proteins are irradiated under the environmental conditions prevailing inside an electron microscope. Previous irradiation experiments with synthetic polypeptides and proteins have shown that the dicarboxylic acids *i.e.* aspartic- and glutamic acid are amongst the most reactive constituents. Alanine and α-aminobutyric acid were, qualitatively, shown to be the radiolytic transformation products of poly-D-aspartic acid and poly-L-glutamic acid respectively [4]. We have suggested a decarboxylation mechanism which we try to substantiate in this communication by presenting the results of a more detailed product analysis of irradiated poly-L-glutamic acid.

Materials and Methods

Because of the volatility of some of the anticipated decarboxylation products the irradiation experiments described in this communication were not performed in an electron microscope. Instead solid samples (0.05 g) of poly-L-glutamic acid (Sigma, St. Louis, USA) were placed in evacuated glass tubes fitted with two stopcocks and irradiated at room temperature in a Nuclear Engineering Ltd. Co-60 gamma source. Doses between 6.2 and 218 Mrad were delivered to the samples at a dose rate of 3.6 Mrad/h. A Fricke dosimeter was used for dosimetry. Gas chromatographic analysis (Perkin Elmer 900 and Carlo Erba 2900) was carried out as described previously [5]. Amino acids were analysed with a Biotronik LC 6000 amino acid analyser after hydrolyzing the non-volatile material (6 N HCl + phenol; 24 h at 110 + 2 °C).

Results and Discussion

The only amino acid transformation product found in significant amounts upon irradiation of poly-L-glutamic acid is α-aminobutyric acid (see Table I); it obviously results from a decarboxylation of glutamic acid side chains. The amount of complementary products detected, like CO_2 or CO, is, initially at least, surprisingly low. Table I shows that the total yield of CO_2 and CO becomes roughly equal to that of α-aminobutyric acid only at very high doses. This might be due to a trapping of the gaseous irradiation products in the poly-L-glutamic acid sample. Only after saturation and/or extensive destruction of the crystals at higher doses CO_2 and CO begin to escape and the total yield observed will become equal to that of α-aminobutyric acid.

Table I. Products found upon irradiation of poly-L-glutamic acid.

Dose	Radiolysis products [μmol]		
[Mrad]	CO	CO_2	α-aminobutyric acid
6.2	0.04	0.31	0.68
14.5	0.07	0.15	1.43
36.3	0.30	0.35	2.60
58.1	0.30	0.53	3.80
72.6	0.13	0.30	6.02
100.0	0.53	0.21	7.86
217.8	3.00	5.20	9.37

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* Part I in this series is Ref. 4

Reprint requests to Priv. Doz. Dr. W. Baumeister.

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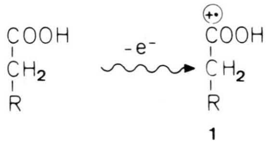
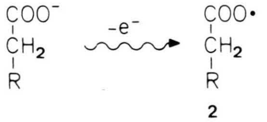
We have previously proposed a radical cation mechanism for the irradiation induced decarboxylation of the glutamic acid side chains leading to the formation of CO_2 and α-aminobutyric acid [4]. The detection of CO_2 now provides strong support for this mechanism. The primary action of ionizing radiation may involve ejection of an electron from the side chains of poly-L-glutamic acid hence leading to radical cation 1 or carboxyl radical 2 depending on the ionization state of the carboxylic group.

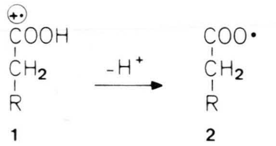


$$\mathrm{R{-}CH_2{-}COOH} \xrightarrow{-e^-} \underset{\mathbf{1}}{\mathrm{R{-}CH_2{-}\overset{+\bullet}{COOH}}} \qquad (1)$$



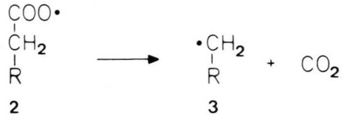
$$\mathrm{R{-}CH_2{-}COO^-} \xrightarrow{-e^-} \underset{\mathbf{2}}{\mathrm{R{-}CH_2{-}COO\bullet}} \qquad (2)$$

The radical cation 1 could deprotonate also forming radical 2.



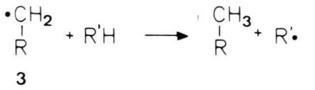
$$\underset{\mathbf{1}}{\mathrm{R{-}CH_2{-}\overset{+\bullet}{COOH}}} \xrightarrow{-H^+} \underset{\mathbf{2}}{\mathrm{R{-}CH_2{-}COO\bullet}} \qquad (3)$$

Radical 2 can easily lose CO_2.



$$\underset{\mathbf{2}}{\mathrm{R{-}CH_2{-}COO\bullet}} \longrightarrow \underset{\mathbf{3}}{\mathrm{R{-}CH_2\bullet}} + \mathrm{CO_2} \qquad (4)$$

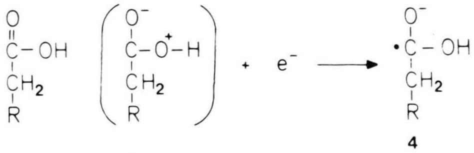
Radical 3 can subsequently recombine with another radical forming a cross-linked product or abstract an hydrogen atom from another glutamic acid residue (R′H).



$$\underset{\mathbf{3}}{\mathrm{R{-}CH_2\bullet}} + \mathrm{R'H} \longrightarrow \mathrm{R{-}CH_3} + \mathrm{R'\bullet} \qquad (5)$$

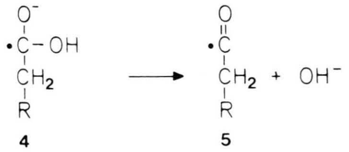
As a result of reaction (5) an altered side chain is formed yielding upon HCl-hydrolysis α-aminobutyric acid.

Similarly the formation of CO can be rationalized via a radical anion mechanism. In the first step the addition of a secondary electron ejected from one of the carboxylic groups to the positive center of another carboxylic group may take place:



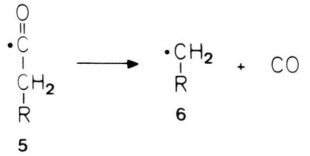
$$\mathrm{R{-}CH_2{-}C({=}O){-}OH} \;\left(\mathrm{R{-}CH_2{-}C(O^-){-}\overset{+}{O}{-}H}\right) + e^- \longrightarrow \underset{\mathbf{4}}{\mathrm{R{-}CH_2{-}\bullet C(O^-){-}OH}} \qquad (6)$$

The formation of a radical anion through the reaction of a hydrated electron with carboxylic acids is known to proceed with diffusion controlled rate constants [6]. The radical anion could subsequently dehydroxylate.



$$\underset{\mathbf{4}}{\mathrm{R{-}CH_2{-}\bullet C(O^-){-}OH}} \longrightarrow \underset{\mathbf{5}}{\mathrm{R{-}CH_2{-}\bullet C{=}O}} + \mathrm{OH^-} \qquad (7)$$

Radical 5 can easily split off CO.



$$\underset{\mathbf{5}}{\mathrm{R{-}CH_2{-}\bullet C{=}O}} \longrightarrow \underset{\mathbf{6}}{\mathrm{R{-}CH_2\bullet}} + \mathrm{CO} \qquad (8)$$

Radical 6 also via reaction (5) will form an α-aminobutyric acid residue.

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