Radiation Damage to Polypeptides and Proteins in the Solid State: Changes in Amino Acid Composition *

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* *Dedicated to* Prof. H. Zahn, Aachen, *on the occasion of his 65th birthday.*

Radiation Damage to Proteins, Solid State Radiolysis, Changes in Amino Acid Compositions, Electron Microscopy

Thin layers of synthetic homopolypeptides (poly-α-Ala, -Arg, -Asn, -Asp, -Glu, -His, -Lys and -Tyr) and proteins (myoglobin, concanavalin A, trypsin-inhibitor) were irradiated under solid state conditions in an electron microscope with 100 keV electrons. Radiolytic changes were investigated by amino acid analysis. The results are discussed in terms of the relative radiosensitivities of the constituent amino acids, and possible topochemical effects on the sensitivity pattern emerging. An attempt is also made to trace at least some of the predominant pathways of amino acid transformation, namely the production of alanine and α-aminobutyric acid.

Introduction

Present knowledge about solid state radiolytic changes in biopolymers is still quite fragmentary in spite of the fact that there are several important areas in molecular biology where such knowledge is urgently needed. Inter alia radiation damage is a fundamental problem in electron microscopy, preventing us from taking full advantage of today's high resolution instruments [1]. Also the prospects for utilizing alternative short-wavelength radiations, potentially useful for molecular structure determination, are seriously hampered by radiation damage [2]. Our interest is primarily focussed on evaluating the kind and amount of structural deteriorations that occur, inevitably or not, in proteins upon exposure to energetic (100 keV) electrons [3]. This is not just a matter of academic interest but is indeed of considerable practical relevance in assessing the trustworthiness of structural information gathered under such hostile environmental conditions and in giving guidelines for "non-destructive" low dose microscopy notwithstanding that these have to be compromised in practice. Such knowledge may also prove helpful in designing, in a sensible way remedial protective measures.

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Apart from the specific environmental conditions prevailing inside an electron microscope (solid state, vacuum) it is the exceedingly high dose delivered to the specimen which distinguishes the situation from that traditionally encountered in radiation chemistry and radiation biology. Molecular radiation biology is usually concerned with doses leading to inactivation (typically a dose between 1 and 50 Mrad for inactivation of enzymes), which is three to four orders of magnitude lower than standard doses in electron microscopy. A dose of 500 e^-/nm^2 – supposing a LET of 4 keV/μm for a 100 keV electron beam traversing a thin layer of carbonaceous material – corresponds to 3000 Mrad; this is regarded as a low dose in "molecular microscopy". To effect even a moderate reduction of dose below this level of irradiation – and this can hardly be more than a factor of 10 in practice-necessitates already sophisticated image processing techniques to restore the "readability" of structural records suffering from quantum noise.

This investigation is part of a broader program aimed at obtaining a detailed picture of radiation damage to proteins on a chemical level, *i.e.* it is concerned with the manifestations of damage rather than with the cascade of physical and physicochemical processes finally leading to permanent chemical changes. Changes in amino acid composition are only one facet of structural damage. How-

ever, since the unique 3-D structure adopted by a protein is, in a given environment, dictated by its amino acid sequence, some knowledge about primary structure changes is indeed indespensible in order to understand reorganisations on the secondary and tertiary structure level.

A first "pilot" study [4] has shown that, as one might expect, radiolytic changes in proteins are not distributed randomly along the polypeptide chain. In fact, amino acids fall roughly into three categories: some amino acid residues remain obviously unaffected, the majority shows a more or less rapid decay and some show a significant increase in amount probably as a result of amino acid transformations. This investigation seeks to establish a broader base for the radiosensitivity pattern found with catalase [4], either to confirm or to modify it, by repeating the irradiation experiments with three proteins of distinctly different secondary structure: myoglobin, which is almost exclusively α-helical, concanavalin A, which has predominantly β-structure and trypsin-inhibitor with a "mixed" conformation. Also an attempt is made to trace at least the most important transformation pathways in experiments with simple synthetic homopolypeptides.

Materials and Methods

Concanavalin A and myoglobin were purchased from Serva, Germany. Trypsin-inhibitor (Trasylol®) was kindly provided by the Bayer AG (Wuppertal-Elberfeld, Germany). Polypeptides were obtained from Sigma (St. Louis, Mo., USA): poly-L-asparagine (MW = 8000), poly-L-arginine (MW = 60000), poly-L-aspartic acid (MW = 14000) and poly-L-tyrosine (MW = 47000) and from Miles-Yeda (Karkakee, Illinois, USA): poly-L-histidine (MW = 11000), poly-D-lysine · HBr (MW = 120000), poly-D-glutamic acid (MW = 47000), poly-DL-alanine (MW = 3800) and poly-L-proline (MW = 1500–15000). All reagents used in amino acid analysis were analytical grade.

Aqueous solutions of the polypeptides and the proteins were spread on one side of 0.02 mm thick gold foils which were made hydrophilic by plasma cleaning to give homogeneous films. 50 µg of the polypeptides and 300 µg of the proteins were deposited on the gold foils which gave films thin enough (~ 0.2 µm) to be "transparent" for 100 keV electrons. These coated foils were irradiated with 100 keV electrons, in the image plane of an electron microscope (Siemens Elmiscope Ia, vacuum 10^{-5} Torr). By irradiating the three protein samples simultaneously it was ensured that the doses were exactly the same. The dosimetric considerations took into account the effect of electron backscattering from the gold foil, which is too thick to be traversed by 100 keV electrons. For details of dosimetry see [5, 6]. The proteins and polypeptides were hydrolyzed on the gold foils in vacuum sealed tube (6 N HCl + phenol) for 24 h at 110 ± 2 °C. Aliquots of hydrolyzates were analyzed with a Biotronik LC 6000 amino acid analyzer equipped with an automatic sampler and Biotronik integrating device. The separation of amino acids was carried out on a polystyrene resin DC 4 A column (Durrum, USA) with 5 different buffers. As an internal standard, norleucin (100 µg) was added before the hydrolysis of proteins; no internal standard was used in the hydrolysis of polypeptides.

Results and Discussion

Radiosensitivities of amino acids in proteins

Myoglobin, concanavalin A and trypsin-inhibitor have been irradiated with electron doses up to 240 e^-/nm^2. The changes in amino acid composition are summarized in Table I. No corrections have been made for losses due to HCl hydrolysis or for imcomplete hydrolysis, since all samples were hydrolyzed under identical conditions. Obviously the amino acid compositions changes quite drastically over the dose range investigated. The majority of amino acids decreases substantially, a few (glycine, alanine) show an increase and with all three proteins a new amino acid, α-aminobutyric acid, which is not contained in the native non-irradiated proteins, is found. This increase of glycine and alanine as well as the production of α-aminobutyric acid must obviously be ascribed to transformations of more complex into simpler amino acids; possible transformations pathways will be discussed in some detail later.

Those amino acids which decrease only moderately (< 30%) over the dose range investigated are: valine, leucine, isoleucine and phenylalanine. Those which decrease drastically (> 30%) are: glutamine/glutamic acid, asparagine/aspartic acid, lysine, arginine, histidine, serine, threonine and tyrosine. Sulphur containing amino acids which are present in all three

Table I. Changes of amino acid composition[a] of proteins upon irradiation.

Amino acid	Myoglobin Dose [e^-/nm^2]					Concanavalin A Dose [e^-/nm^2]					Trypsin-Inhibitor Dose [e^-/nm^2]				
	0	30	60	120	240	0	30	60	120	240	0	30	60	120	240
Asx	49	41	30	23	18	99	81	73	55	43	58	53	49	44	39
Glx	109	94	71	58	45	42	37	32	25	26	40	35	32	27	24
Thr	33	31	25	22	18	62	49	43	33	24	34	33	33	28	25
Ser	33	34	26	24	20	84	71	62	51	41	15	14	14	12	16
Gly	65	65	56	54	59	58	57	62	64	78	78	68	66	64	62
Ala	89	93	82	83	80	57	58	60	63	63	64	64	65	64	62
Abu	–	12	15	24	32	–	3	5	9	10	–	2	3	6	8
Val	43	43	37	35	32	48	44	42	40	38	14	13	15	13	13
Ile	43	43	36	34	30	43	38	35	33	27	18	17	17	15	14
Leu	94	93	78	74	66	58	53	48	44	38	24	23	23	21	19
Tyr	17	17	14	13	11	24	20	17	16	13	45	41	37	33	30
Phe	34	33	27	25	23	37	32	30	28	24	46	43	41	39	36
Lys	99	87	71	57	46	38	25	21	16	12	45	38	36	26	24
His	61	56	48	43	37	19	15	14	13	15	–	–	–	–	–
Arg	27	26	22	19	16	21	19	17	14	13	67	60	58	52	48
% Recovered[b]	100	94.5	78.3	70.9	62.9	100	86.8	79.6	70.0	62.2	100	91.6	88.7	79.9	75.4

[a] These values are in nanomoles.
[b] Calculated without including α-aminobutyric acid and increases in alanine and glycine.

proteins only in very small amounts (except for cysteine in trypsin-inhibitor) have not been analysed. Taking into account the extended dose range in the present study this radiosensitivity pattern is almost identical to the one reported earlier for catalase [4]. The pattern is, despite some clear differences between the different protein species, correlated with the chemical structure of the amino acid side chains.

As transformation products only amino acids with short aliphatic side chains are obtained. The long side chains of aliphatic amino acids are fairly resistant: not more then 30% of them is lost when the proteins are exposed to 240 e^-/nm^2. The aromatic amino acids are of intermediate sensitivity, tyrosine with its additional phenolic hydroxyl group being slightly more sensitive than phenylalanine. The hydroxyamino acids, serine and threonine, the dicarboxylic amino acids and their amides, glutamine/glutamic acid, asparagine/aspartic acid and the amino acids carrying basic functions are of roughly equal sensitivity. Among the basic amino acids lysine clearly ranks as the most sensitive one. The decrease of these amino acids amounts up to 60% over this dose range.

Radiosensitivities of amino acids in homopolypeptides

The main purpose of the irradiation studies with the homopolypeptides was to trace possible precursors for the transformation products glycine, alanine and α-aminobutyric acid. However, as a sideline it is interesting to compare the radiosensitivities of some amino acids in these synthetic homopolypeptides and in the proteins respectively.

Table II. Complete amino acid composition of the three proteins under investigation and assignment of probabilities to be located in α-helical (P_α) or β-structure (P_β) regions (from ref. 9).

Amino acids	Myoglobin [%]	Concanavalin A [%]	Trypsin-Inhibitor [%]	P_α	P_β
Glu	8.5	5.1	3.5	1.53	0.26
Ala	9.8	7.6	10.3	1.45	0.97
Leu	11.4	8.0	3.5	1.34	1.22
His	7.2	2.5	–	1.24	0.71
Met	1.3	0.8	1.7	1.20	1.67
Gln	3.9	2.1	1.7	1.17	1.23
Trp	1.3	1.7	–	1.14	1.19
Val	4.5	7.2	1.7	1.14	1.65
Phe	4.5	4.2	6.9	1.12	1.28
Lys	12.4	4.6	6.9	1.07	0.74
Ile	5.9	6.8	3.5	1.00	1.60
Asp	4.6	8.4	3.5	0.98	0.80
Thr	4.6	8.0	5.1	0.82	1.2
Ser	3.3	12.7	1.7	0.79	0.72
Arg	1.3	2.5	10.3	0.79	0.90
Cys	–	8.4	10.3	0.77	1.3
Asn	2.0	5.1	5.1	0.73	0.65
Tyr	1.3	3.0	6.9	0.61	1.29
Pro	2.6	4.0	6.9	0.59	0.62
Gly	9.8	6.8	10.3	0.53	0.81

Table III. Changes in homo-polypeptides[a] upon irradiation.

Polypeptide	Content of constituent amino acids[b]		Transformation products[b]			
			Ala		Abu	
	75 e^-/nm^2	150 e^-/nm^2	75 e^-/nm^2	150 e^-/nm^2	75 e^-/nm^2	150 e^-/nm^2
Poly-L-Glu	40	18	–	–	20	16
Poly-D-Asp	57	27	10	13	–	–
Poly-L-Pro	54	34	–	–	–	–
Poly-L-Arg	68	48	–	–	–	–
Poly-L-Asn	73	68	–	–	–	–
Poly-DL-Ala	80	53	–	–	–	–
Poly-L-Tyr	82	76	–	–	–	–
Poly-L-His	84	78	–	–	–	–
Poly-L-Lys	89	63	–	–	–	–

[a] For irradiation of a 50 µg homo-polypeptides.
[b] All values are given in per cent of nonirradiated homo-polypeptides.

In Table II the homopolypeptides are listed according to the relative radiosensitivities of their constituent amino acids. The amino acids with acidic functions, glutamic acid and aspartic acid are extremely sensitive, more sensitive indeed than in proteins. A dose of 150 e^-/nm^2 leads to a loss of ~ 40% of the glutamic acid/glutamine residues in proteins and to a loss of ~ 80% in poly-L-glutamic acid. Since we are unable to distinguish between aspartic acid and asparagine, as well as between glutamic acid and glutamine in proteins – we obtain only averaged data for these pairs – it is interesting to note that in homopolypeptides the dicarboxylic acids are obviously more sensitive than their amides. This is observed at least with poly-D-aspartic acid and poly-L-asparagine (Table III). It means that figures obtained for glutamic acid/glutamine and aspartic acid/asparagine might well give an underestimate for the dicarboxylic acids and overestimate for the corresponding amides. This cannot account, however, for the drastically increased sensitivity of the dicarboxylic acids in homopolypeptides.

Another noticeable result is the relatively high sensitivity of alanine in poly-DL-alanine; a dose of 150 e^-/nm^2 leads to a loss of about 50% of the alanine residues. Therefore it appears to be dangerous to conclude from the data given in Table I that alanine residues remain unaffected in proteins upon irradiation, as the net-increase of alanine in concanavalin A might suggest. Amino acid transformation might indeed mask the damage to original alanine residues. Supposing on the other hand that in proteins alanine is roughly as sensitive as the other aliphatic amino acids (valine, leucine and isoleucine), which because of their complex side chain stucture can hardly originate from any other precursor amino acid, the net-increase of alanine particularly in concanavalin A can not be regarded as a proper measure for the abundance of transformation reactions. It gives only a minimum figure, which is likely to be an underestimate.

Quite surprising is, at a first glance, the relatively low rank in terms of radiosensitivity of the basic amino acids, in particular of lysine. It should be noted however, that the absolute figures (loss of 35–50% of the original residue at a dose of 150 e^-/nm^2) do compare quite well with those measured with proteins. So it is probably not a decrease in the sensitivity of the basic amino acids but an increase in the sensitivity of the acidic ones, which makes the radiosensitivity pattern to appear so different from that found in proteins.

Variations in radiosensitivity

Apart from these quite obvious differences in the radiosensitivity pattern of homopolypeptides and complex proteins, there are also remarkable variations between different protein species, *i.e.* the radiosensitivity of a given amino acid may differ significantly from one protein to another. Fig. 1 clearly shows that some amino acids, like valine or tyrosine *e.g.* behave fairly familiar in all three proteins investigated, while others behave rather dif-

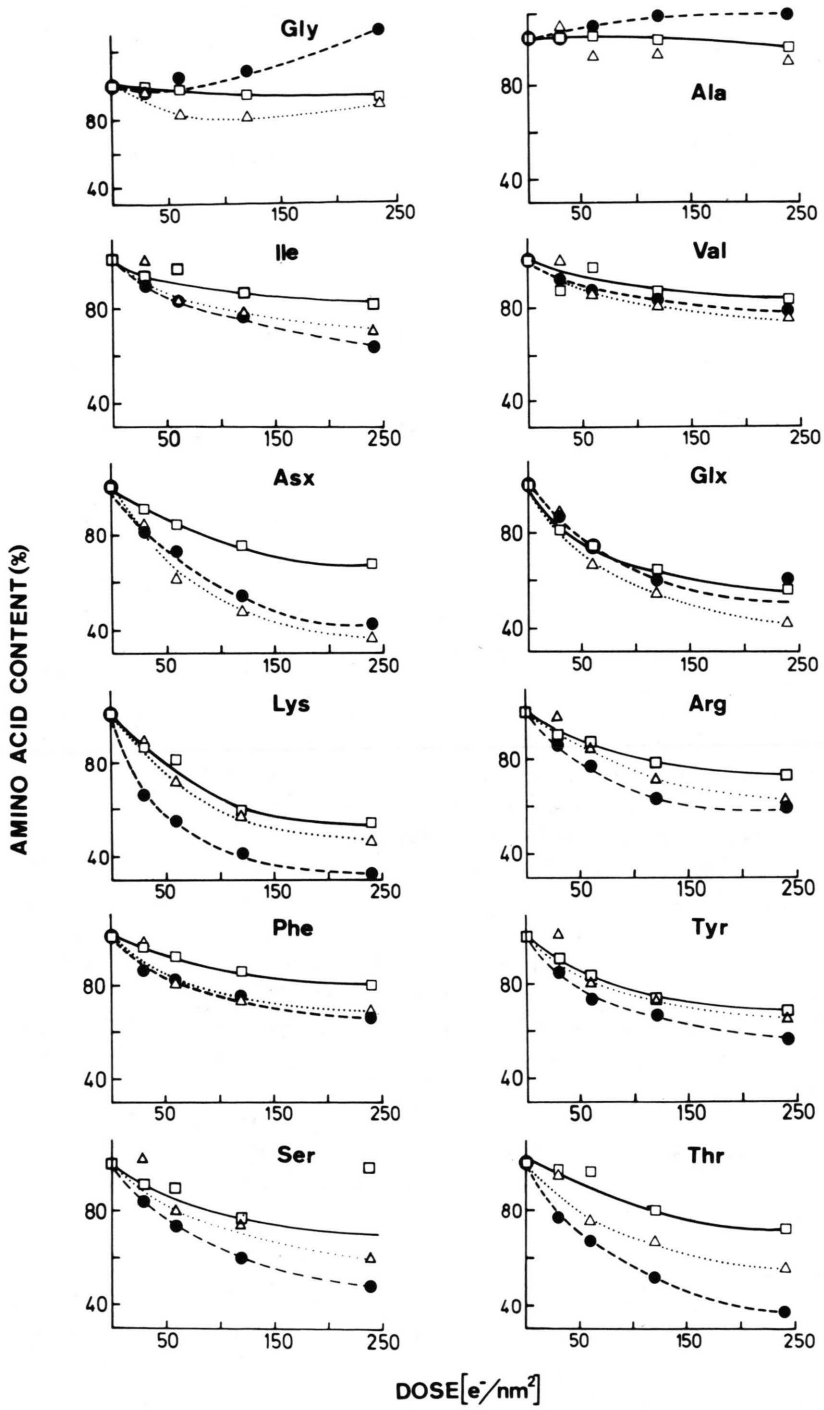


Fig. 1. Changes in amino acid composition in (●) concanavalin, (△) myoglobin and (□) trypsin-inhibitor upon irradiation.

ferent chemical environments provided by these proteins. This we call the "topochemical" effect.

It emerges as a sort of a general pattern that the radiosensitivity of a given amino acid is always lower in trypsin-inhibitor than in myoglobin and in concanavalin A (Fig. 1). As a consequence, also the total number of amino acids lost or transformed is particularly low with trypsin-inhibitor (see also Table I) and this is paralleled by an unusually low mass loss (see below). We are unable, at present, to rationalize why trypsin-inhibitor behaves so remarkably different from the other proteins. We should like to point, however, to the unusually high proportion of cysteine residues in this molecule (Table II).

When comparing myoglobin and concanavalin A the differences appear to be less clear. At a given dose the total number of amino acids lost or transformed is approximately the same for both proteins (see Tables I and V). It is interesting to observe, however, that the majority of amino acids is more liable to radiation damage in concanavalin A than in myoglobin with two quite remarkable exceptions: glutamic acid/glutamine and aspartic acid/asparagine. Some thoughts and speculations trying to explain this behaviour in terms of topochemical effects are given below.

Topochemical effects: Some facts and speculations

It is obvious from the experimental data presented so far that damage to the protein primary structure is not a random process. The constituent amino acids decrease at different rates, despite their stochastic interaction with the impinging radiation energy. Intramolecular energy transfer and the selectivity of secondary chemical reactions are probably the main mechanisms responsible for the radiosensitivity patterns emerging. These patterns, although having several features in common, are subject to some variation from one protein species to another. In view of the otherwise constant "environmental" conditions it seems logical to ascribe these variations to the different chemical environments provided by the proteins themselves. At a first glance it seems that the environmental situation is on an average rather constant within large globular proteins. We feel, however, that there are differences in composition and spatial organization, which could well act as topochemical factors, decreasing or increasing the sensitivity of the constituent amino acids.

Notwithstanding the general importance of irradiation experiments with mono-amino acid systems or homopolypeptides, their relevance for proteins is rather limited. It is dangerous to predict on basis of such experiments the behaviour of a given amino acid in a more complex molecule; proteins behave not like a non-interacting mixture of amino acids. The drastically increased sensitivity of the dicarboxylic acids in homopolypeptides is a particular striking example giving also a first hint at a possible topochemical effect. The most obvious difference between the three proteins investigated and poly-L-glutamic acid is of course that the latter is made up exclusively of glutamic acid residues. Hence it is tempting to speculate that it is in this particular case the high concentration of glutamic acid which increases the radiosensitivity. A close proximity of glutamic acid residues is in fact likely to accelerate the radiolytic destruction, supposing that the transformation mechanism delineated below applies.

We realize that this is an extreme situation and the crucial question is, of course, whether or not effects of this kind are likely to play a significant role also in proteins. The overall concentration of a given amino acid may vary considerably between different protein species (see *e.g.* Table II) but only rarely exceeds 10%. Locally, however, the concentration may be much higher. Glutamic acid, *e.g.* has a high probability to be located in α-helical regions – it is in fact the strongest α-helix "former" following the terminology of Chou and Fasman [7] – while some other amino acids are "forbidden" there. Such clustering might well increase the sensitivity relative to situations where the mean distance between the glutamic acid residues is larger. In myoglobin, which is predominantly α-helical, a clustering of glutamic acid residues is in fact observed [8] and this could explain the significantly increased sensitivity of this amino acid in myoglobin (Fig. 1). This sensitivity is more adequately reflected by the increase in α-aminobutyric acid content, the main transformation product (Table I) of glutamic acid (see below), than by the decrease in glutamic acid-glutamine content.

This clustering of glutamic acid residues is only a first example of a topochemical effect and further experiments are needed to substantiate our proposal. We could imagine a vast number of possible topochemical effects, governed by composition as well as by conformation, capable of modyfying the radiosensitivity pattern of proteins.

Mass loss and mass balance

The data given in Table III show that the total amount of amino acids lost is far from being balanced by the total amount of transformation products found. At a dose of 240 e^-/nm^2 the transformation products account only for 6% (trypsin-inhibitor) up to 15% (concanavalin A) of the radiolytic changes. The deficit must probably be ascribed to various sources. One possible source is certainly mass loss which is a well established fact in electron microscopy. Many low molecular weight fragments liberated by bond scissions are volatile under the vacuum conditions prevailing in electron microscopes; these fragments evaporate reducing the mass of their parent molecules. It has been shown by means of autoradiography that virus particles and ribosomes lose about 15% of their original mass during a 30 sec exposure to a 100 keV beam [9]; similar figures were obtained for several other proteinaceous molecular assemblies by direct mass determination in a scanning transmission electron microscope [10]. We have employed ATR-infrared spectroscopy to determine the amount of mass loss for a number of proteins [11]. The integrated absorption of the amide I and II bands can be taken as an approximate measure for the residual mass.

Table IV summarizes the mass loss figures relevant in the context of the present paper. While myoglobin and concanavalin A both lose about 15% of their original mass when irradiated with 214 e^-/nm^2, mass loss is significantly lower with trypsin-inhibitor (5%). The only reasonable explanation we can offer at the moment for this untypical behaviour of the trypsin-inhibitor molecule is that the high cysteine content (see Table II) may favour intramolecular crosslinking and it is likely that a high incidence of crosslinking counteracts mass loss. This is at least an interesting speculation worth to pursue because of its implications for practical microscopy.

Table IV. Mass loss data as derived from ATR-infrared spectroscopy [a].

Dose [e^-/nm^2]	Myoglobin [%]	Concanavalin A [%]	Trypsin-Inhibitor [%]
0	100	100	100
53	89	96	96
107	87	92	95
214	84	85	95

[a] Decrease of integrated absorption of the amide I and II bands, given in per cent of nonirradiated specimen.

Table V. Mass balance at a dose of 240 e^-/nm^2.

	Myoglobin %	Concanavalin A %	Trypsin-Inhibitor %
Amino acid recovered	63	62	75
Transformation products found:			
α-Aminobutyric acid	4	2	2
Glycine	–	3	–
Alanine	–	1	–
Total	67	68	77
Mass loss as derived from IR [a]	16	15	5

[a] For a dose of 214 e^-/nm^2 (from Table II).

Taking into account the transformation products and the mass loss data as derived from infrared spectroscopy we arrive at an almost equal deficit in the "mass balance" of all three proteins amounting to ~ 18% (as can be calculated from (Table V). Ninhydrine-negative and/or heavily crosslinked non-hydrolyzable products could account for this deficit. Further investigations are in progress to identify and quantify them at least partially.

Transformation pathways

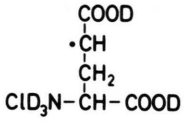
Amino acid transformation appears to be a common phenomenon in the solid state radiolysis of proteins. It provides an adequate explanation for the formation of α-aminobutyric acid which is not contained in native proteins but usually found in appreciable amounts upon irradiation (see also [4, 12]) as well as for the increasing amounts of glycine and alanine. Theoretically many amino acids contained in proteins could be the precursors of these three transformation products and a multitude of transformation pathways has in fact been observed when amino acids, small synthetic peptides and high molecular weight homopolypeptides were irradiated in aqueous solution [13–15]. Many of these transformation reactions are unlikely, however, to occur also under solid state conditions.

In order to trace the precursors of α-aminobutyric acid, alanine and glycine we have selected 9 polypeptides, the most likely candidates for transformation reactions, and have irradiated them under the

same conditions as the proteins. The results, which are summarized in Table III, suggest two main transformations, the formation of alanine from aspartic acid and of α-aminobutyric acid from glutamic acid. Both transformations could be explained in terms of a side chain decarboxylation. A possible mechanism is proposed below. Minor amounts of glycine have been found upon irradiation of poly-L-tyrosine, but there are several other potential precursors for this simplest of all amino acids.

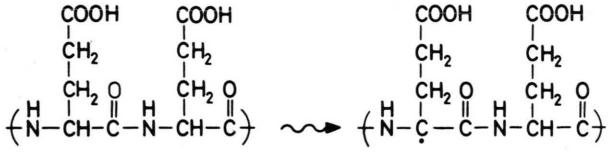
Mechanism of decarboxylation: A proposal

The transformation of the dicarboxylic acids into α-aminobutyric acid and alanine must obviously proceed via a decarboxylation of their side chains. It has been shown previously for x-ray irradiated L-glutamic acid · DCl crystals that an unpaired electron is likely to be localized at the carbon atom adjacent to the carboxyl group remote from the amino group [16]. ESR studies established the existence of radical **1**.



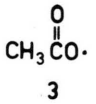
1

On the other hand ESR studies with poly-L-glutamic acid revealed the existence of a different type of radical upon irradiation [17] and the most probable radical, which involves the polypeptide main chain, is given in **2**.



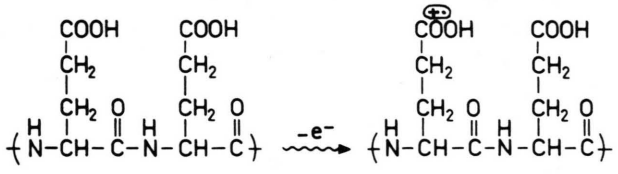
2 (1)

The production a α-aminobutyric acid, on the other hand, is not likely to originate from radicals **1** or **2**, since it requires a radical rearrangement which is energetically improbable. Previous ESR studies in aqueous solutions [18] have shown that the decarboxylation of acetic acid proceeds via an acetoxyl radical **3**.



3

Radicals similar to **3** are known to be involved in the electrochemical oxidation of carboxylic acids which is known as Kolbe reaction [19]. Similarly the decarboxylation of poly-L-glutamic acid can probably be rationalized via carboxyl radicals. The primary action of ionizing radiation involves ejection of an electron from the substrate, *i.e.* among other molecular groups probably also from the carboxyl and/or carboxylate groups of the poly-L-glutamic acid side chains. Reactions **2** and **3** would lead to formation of radical cation **4** and/or carboxyl radicals **5**.



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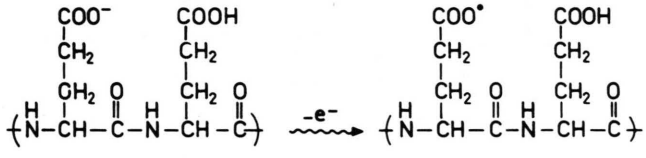
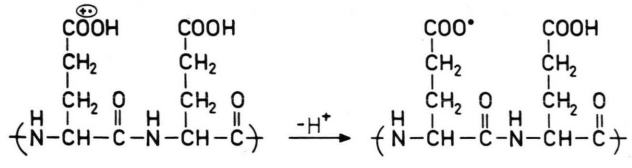


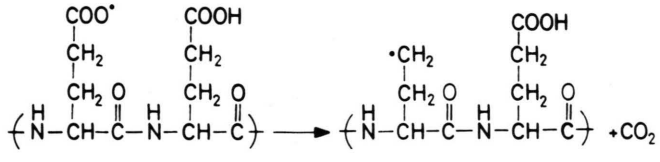
5 (3)

The radical cation **4** could deprotonate also forming radical **5**.



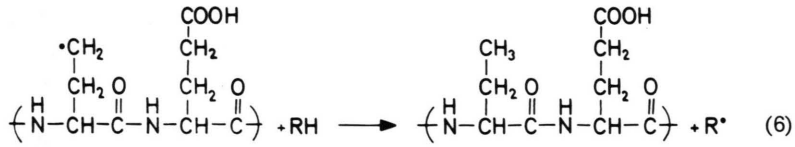
4 5 (4)

Radical **5** will readily lose CO_2.



5 6 (5)

The production of CO_2 is well established for irradiated aliphatic carboxylic acids [19–22]. Radical **6** can subsequently recombine with another



6

radical forming a cross-linked product or abstract an H atom from another amino acid residue (RH).

As a result of reaction **6** an altered side chain is formed yielding upon acid hydrolysis α-aminobutyric acid. The formation of alanine observed upon irradiation of poly-D-aspartic acid can be explained by an analogous mechanism.

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[1] W. Baumeister, Cytobiologie **17,** 246 (1978).
[2] J. R. Bredlove and G. T. Trammel, Science **170,** 1310 (1970).
[3] W. Baumeister and J. Seredynski, Proc. 9th Int. Congr. on Electron Microscopy, Toronto, **Vol. III,** 40 (1978).
[4] W. Baumeister, M. Hahn, J. Seredynski, and L. M. Herbertz, Ultramicroscopy **1,** 377 (1976).
[5] M. Hahn, J. Seredynski, and W. Baumeister, Proc. Nat. Acad. Sci. USA **73,** 823 (1976).
[6] W. Baumeister, U. P. Fringeli, M. Hahn, F. Kopp, and J. Seredynski, Biophys. J. **16,** 791 (1976).
[7] P. Y. Chou and G. D. Fasman, Biochem. **13,** 222 (1974).
[8] M. Levitt and J. Greer, J. Mol. Biol. **114,** 181 (1977).
[9] R. E. Thach and S. S. Thach, Biophys. J. **11,** 204 (1971).
[10] A. Engel, Ultramicroscopy **3,** 273 (1978).
[11] J. Seredynski, Ph. D. Thesis, Düsseldorf University, Düsseldorf, Bundesrepublik Deutschland (1979).
[12] P. Alexander and L. D. G. Hamilton, Radiat. Res. **13,** 214 (1960).
[13] F. Friedberg and G. A Hayden, Science **140,** 825 (1963).
[14] J. Kopoldova, J. Rolousek, A. Barbicky, and J. Liebster, Nature **192,** 352 (1961).
[15] W. M. Garrison, Current Topics in Radiation Research, (M. Ebert and A. Howard, eds.) **Vol. 4,** p. 45, North Holland Publishing Co., Amsterdam 1968.
[16] D. J. Whelan, J. Chem. Phys. **11,** 4734 (1968).
[17] R. C. Drew and W. Gordy, Radiat. Res. **18,** 552 (1963).
[18] W. T. Dixon, R. O. C. Norman, and A. L. Buley, Proc. Chem. Soc. **1964,** 3625.
[19] D. C. Nonhebel, J. M. Tedder, and J. C. Walton, "Radicals", p. 46, Cambridge University Press, Cambridge 1979.
[20] A. R. Jones, Radiat. Res. **47,** 35 (1970).
[21] A. R. Jones, Radiat. Res. **48,** 447 (1971).
[22] G. S. Wu and D. R. Howton, Radiat. Res. **57,** 390 (1974).