DEGRADATION OF SOME POLY-α-AMINO ACIDS AND PROTEINS BY THE RADIOMIMETIC SYSTEM Cu^{2+}–H_2O_2

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We wish to report the first evidence for the degradation of poly-α-amino acids by the radiomimetic system Cu^{2+}–H_2O_2 (unstabilized 3% hydrogen peroxide in the presence of trace amounts of Cu^{2+}).

The degradation at room temperature of proteins and peptides by the radiomimetic system Cu^{2+}–H_2O_2, which contains peroxyl and hydroxyl free radicals, has been studied in a few instances.[1] A radiomimetic system is imitative in its activity of ionizing radiations, since both generate hydroxyl free radicals in aqueous systems.

In the present study we determined the effect of the Cu^{2+}–H_2O_2 system on some poly-α-amino acids. Since the results indicated that the nature of the amino acid monomer was important, we extended the study to include proteins of widely differing amino acid compositions. The chemical reactions that are involved in the degradation by both ionizing radiations and the radiomimetic system are complex. Many non-nitrogenous organic products are formed, the specific products depending on the amino acid residue. Ammonia from the peptide linkage is a major product in all cases.[1–3] Since the reaction mixtures were frequently inhomogeneous systems, and therefore determination of the extent of degradation by physical measurements on the solutions was not possible, the amount of ammonia formation was taken as an overall measure of extent of degradation of the polyamide chains.

The results are given in Tables 1 and 2. As a class, the poly-α-amino acids were less readily degraded than the proteins at 21°, but at 37° the amount of degradation became essentially of the same order of magnitude as that of the proteins at the lower temperature. There were great differences among the polyamides with respect to resistance to attack. For example, polyglycine and poly-L-aspartic acid were much more susceptible to degradation than were poly-L-leucine and poly-L-proline. Some proteins (such as zein and ligament elastin) were only slightly attacked, while others (such as serum albumin and γ-globulin) were extensively degraded.

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[1] Phelps, R., Neet, K., Lynn, L., and Putnam, F., *J. biol. Chem.*, 1961, **236**, 96; Curtius, H., Anders, P., Erlenmeyer, H., and Sigel, H., *Helv. chim. Acta*, 1968, **51**, 896; Sigel, H., and Curtius, H., *Experientia*, 1966, **22**, 649.

[2] Hart, E. J., and Platzman, R. L., in "Mechanisms of Radiobiology." (Eds M. Errera and A. Forssberg.) Vol. 1, pp. 93–247. (Academic Press: New York 1961.)

[3] Deasy, C., Broerman, M., Shively, S., Alexander, Sr. A., and Hart, R. V., *J. Am. Leath. Chem. Assoc.*, 1970, **65**, 573.

An explanation for the resistance to attack by the radiomimetic system of some polyamides and the ready degradation of others may be sought. That the solubility of the polyamide in the aqueous system is not of prime importance is shown by

TABLE 1

ACTIVITY OF THE CUPRIC ION–HYDROGEN PEROXIDE SYSTEM ON POLY-α-AMINO ACIDS

All experiments were carried out with 2 mg poly-α-amino acid per ml solution, except in the case of poly-L-proline, where the concentration was 1 mg per ml. Molecular weights as given by the manufacturer were: aspartic acid, 2500–6000; hydroxyproline, 10000–20000; lysine, 3000–5000; valine, 1000–5000; and alanine 2500–10000

Poly-α-amino acid	Nitrogen (%) converted into ammonia		
	21°, 1 day	21°, 4 days	37°, 1 day
Poly-L-aspartic acid	5	5	39
Polyglycine	3	6	58
Poly-L-hydroxyproline	1	3	29
Poly-L-lysine,HBr[a]	1	2	20
Poly-L-leucine	0	1	12
Poly-L-valine	1	1	13
Poly-L-alanine	0	1	13
Poly-L-proline[b]	not determined	not determined	0

[a] Calculations were made on the basis of the peptide nitrogen only. [b] At 32°.

TABLE 2

ACTIVITY OF THE CUPRIC ION–HYDROGEN PEROXIDE SYSTEM ON PROTEINS IN RELATION TO THEIR AMINO ACID COMPOSITION

All experiments were carried out at 21° and with 2 mg protein per ml solution, unless otherwise noted. For amino acid compositions of all proteins except fibrinogen and bovine corium elastin, see Tristram, G., and Smith, R., *Adv. Protein Chem.*, 1963, **18**, 227; for fibrinogen, see Tristram, G., *Adv. Protein Chem.*, 1949, **5**, 83; figures for bovine corium elastin are based on our analyses

Protein	Protein N (%) converted into ammonia				Amino acid composition (polar/apolar residues)
	1 day	3 days	7 days	10 days	
Serum albumin (bovine)	37·4	48·7	59·7	65·0	1·61
γ-Globulin (bovine)	33·1	42·3	53·0	59·7	1·56
Gliadin	26·5	36·5	48·6	55·0	1·45
Fibrinogen (human)	17·8	32·1	43·5	48·9	1·91
Collagen (bovine corium)	[c]	31·0	43·9	48·4	1·20
Ovalbumin (hen)	21·4	31·2	41·7	48·8	1·25
Elastin[a] (bovine corium)	[c]	14·9	25·8	32·2	0·71
Elastin[b] (bovine ligament)	[c]	5·8	12·6	15·5	0·13
Zein	3·0	5·4	9·8	13·3	0·84

[a] Concentration 0·5 mg protein/ml. [b] Concentration 0·9 mg protein/ml. [c] Not determined.

the fact that insoluble fibrous collagen is as readily attacked as the much more soluble ovalbumin. Further, slightly soluble polyglycine is readily degraded. The question whether the helical content is the governing factor must also be answered in the negative. For example, bovine serum albumin and hen ovalbumin have 50

and 45% helical content, respectively,[4] and both are readily attacked, while ligament elastin with only an estimated 10% helical content[5] is very resistant. On the other hand, poly-L-alanine would be expected to have a higher helical content than polyglycine[6] and poly-L-lysine,[7] but it is more resistant to the system. Consequently, the helical content cannot be used as a measure of resistance to the Cu^{2+}–H_2O_2 system.

Hatch[8] has shown that a correlation between the polar nature of the amino acid moieties of proteins and certain of their characteristics can be made. In his study he assigns aspartic acid and its amide, glutamic acid and its amide, lysine, arginine, serine, and threonine to a polar group, and valine, leucine, isoleucine, methionine, proline, and phenylalanine to an apolar group; his reasons for not assigning the remaining amino acids to either group are discussed in his original article.

If the polarity of the amino acid residue in the poly-α-amino acids is considered from this point of view, then it is evident from the present study that those with a polar amino acid residue (aspartic acid and lysine) are more readily attacked by the Cu^{2+}–H_2O_2 system than those with an apolar residue (valine, leucine, and proline).

In order to test whether this generalization would carry over to the proteins, the ratio polar/apolar residues was calculated on the basis of the Hatch categories. The results (Table 2) show that those proteins with a high ratio of polar to apolar residues (1·20–1·91) are readily attacked by the system, while those with a low ratio (0·13–0·84) are resistant. Apolar amino acid residues in a protein therefore appear to protect the protein against attack by the radiomimetic system. The reason for the resistance probably resides in the fact that the conformation of polyamides are influenced by the hydrophobic bonding possible with apolar residues.

Implications of this conclusion in the radiation chemistry of polyamides, where the active agents are also free radicals, are immediately evident.

Experimental

All poly-α-amino acids and proteins used were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, except zein, gliadin, and fibrinogen, which were obtained from Mann Research Laboratories, New York, and fibrous collagen, skin elastin, and ligament elastin, which were prepared by the authors. The fibrous collagen was obtained from bovine corium by sequential extraction with 5% NaCl, H_2O, ethanol, and acetone.[9] Skin elastin and ligament elastin were obtained by autoclaving in water bovine corium and ligamentum nuchae, respectively. The radiomimetic system was freshly prepared 3% unstabilized H_2O_2 containing trace amounts of $CuSO_4$ (5×10^{-4}M). Ammonia evolved from the system was determined by acidimetry after the system had been treated with platinum black to decompose excess peroxide and then made alkaline. Conditions used in the experiments are shown in the Tables.

Acknowledgment

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[5] Mammi, M., Gotte, L., and Pezzin, G., *Nature*, 1968, **220**, 371 and 1970, **225**, 380.
[6] Scheraga, H., *Chem. Rev.*, 1971, **71**, 195.
[7] Fasman, G., "Poly-α-Amino Acids." p. 539. (Marcel Dekker: New York 1967.)
[8] Hatch, F., *Nature*, 1965, **206**, 777.
[9] Deasy, C. L., *J. Am. Leath. Chem. Assoc.*, 1967, **62**, 258.