INACTIVATION OF DESOXYRIBONUCLEASE I BY X-RAYS

IV. CHANGES IN AMINO ACID COMPOSITION AND ULTRAVIOLET LIGHT ABSORPTION INDUCED BY IONIZING RADIATION*

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INTRODUCTION

It has been well established by numerous investigators that proteins when exposed to ionizing radiation undergo physical and chemical changes resulting in the modification of molecular structure and biological activity. The pertinent literature on this subject has been reviewed recently by BARRON *et al.*[1]. Although numerous studies have been carried out concerning the radiation-induced changes in proteins, such changes have not been correlated with biological activity. In connection with studies on the effect of ionizing radiations on desoxyribonuclease I (DNase I) by one of the authors (S.O.)[2,3], experiments have been carried out to determine simultaneously the enzymic activity and amino acid composition of the enzyme before[4] and after irradiation of aqueous solutions with X- and γ-rays. These experiments will be described in this paper.

METHODS AND MATERIALS

Crystalline DNase I (purchased from the Worthington Biochemical Co.) was assayed by means of the diphenylamine reaction[5]. The amino acid composition of DNase was determined according to the method of MOORE AND STEIN[6,7]. Tryptophan was estimated separately by the method of SPIES *et al.*[8]. Ultraviolet spectra were determined with a Beckman spectrophotometer, Model DU.

The conditions of X-irradiation have been reported previously[3]. In the experiments on the effect of irradiation on the amino acid composition of DNase, the enzyme solution (5 mg per ml) was irradiated with gamma rays from a 670-curie cobalt source.

RESULTS

The concentration of only five of the amino acid residues present in the DNase molecule was altered as a result of γ-irradiation (Table I). The concentration of all other residues reported previously as constituents of DNase I[4] remained unaltered upon exposure to 680,000 r of γ-rays. It can be seen from Table I that of the five affected amino acid residues, the degree of deamination was greatest in lysine and tryptophan, exceeding 50%, slightly less in isoleucine and histidine, approximately 40%, and only 9% in aspartic acid.

In order to ascertain whether the loss of amino acid could be correlated with the radiation dose and the decrease in enzymic activity, the concentration of tryptophan

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and the enzymic activity were determined simultaneously after irradiation of aqueous solutions with different doses of γ-rays. Table II shows that the concentration of tryptophan as well as the enzymic activity decreased with increasing radiation doses.

TABLE I

CONCENTRATION OF AMINO ACID RESIDUES ALTERED BY IRRADIATION OF DNASE I

Amino acid	*Number of amino acid residues per one enzyme molecule**	
	Non-irradiated	*Irradiated***
Aspartic acid	65	58
Isoleucine	21	13
Lysine	33	14
Histidine	22	13
Tryptophan	5	2
Ammonia (total)	137	184
Total number of amino acid residues per DNase I molecule	560	496

* The values for the amino acid residues of the irradiated DNase I are the averages of two experiments, whereas comparable values for non-irradiated DNase I are based on four experiments.

** 680,000 gamma roentgens from a 670 curie cobalt source.

TABLE II

CONCENTRATION OF TRYPTOPHAN IN IRRADIATED DNASE*

Radiation dose (r)	*Tryptophan concentration***	*Relative enzyme activity*
0	1.32	100
47,300	1.12	76
142,000	1.00	45
284,000	0.88	8
568,000	0.70	0.3

* 2.5 ml of an aqueous solution of DNase I (5 mg per ml) was irradiated with gamma rays. Separate aliquots (1 ml) were assayed for tryptophan and for enzymic activity.

** The concentration is expressed as g of tryptophan per 100 g of dry protein.

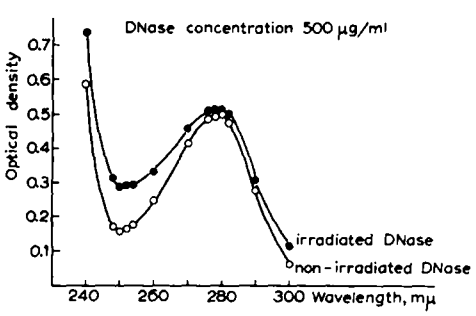

Fig. 1. Ultraviolet absorption spectrum of irradiated and non-irradiated DNase I.

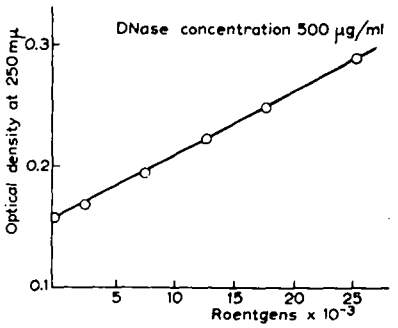

Fig. 2. Correlation of radiation dose and optical density at 250 mμ.

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However, the decrease is linear with dosage in the case of tryptophan concentration and exponential for enzymic activity.

X-irradiation of DNase I in aqueous solution caused a significant increase in ultraviolet absorption (Fig. 1) which was maximal at 250 mμ. At this wavelength, the absorption was related linearly to the dose of radiation (Fig. 2). The enzymic activity, however, decreased in an exponential and inverse fashion with increasing dosage. The pH-dependence of the UV-spectrum was unaffected by irradiation.

Exposure to radiation did not affect the infra-red absorption spectrum, the sedimentation pattern as determined with the analytical ultracentrifuge, or the acid–base titration curve of DNase I solutions.

DISCUSSION

The results presented permit a comparison of the effect of irradiation on various properties of a protein, *i.e.* ultraviolet absorption, amino acid composition and biological activity. It is noteworthy that irradiated DNase I exhibits no change in light absorption at 280 mμ although the tyrosine/tryptophan ratio is 7.43:1.57. Whereas an increase in light absorption at 280 mμ would be expected after irradiation, according to BARRON *et al.*[1], such an increase was observed instead at 250 mμ. Since exposure of DNase I to radiation resulted in a decrease in concentration of only 5 amino acid residues including tryptophan, but not tyrosine and phenylalanine, the observed spectral changes are most likely due to an effect of radiation on tryptophan. This hypothesis is supported by the analytical data in Table II and the decrease in tryptophan residues (Table I). The failure of the spectrum to change at 280 mμ in irradiated DNase I is consistent with the absence of changes in the concentration of tyrosine and phenylalanine residues subsequent to irradiation. It is conceivable that the latter amino acids are present at some distance from the end of the peptide chains or from the surface of the DNase molecule and, for this reason, are inaccessible to oxidizing radicals. Irrespective of mechanism, the observations suggest that the tryptophan residues of DNase I are more radiosensitive than tyrosine and phenylalanine residues. A similar increase in light absorption at 250 mμ has been reported earlier for other irradiated proteins by BARRON AND FINKELSTEIN[9] and, as in the case of DNase I, the light absorption at 250 mμ increases linearly with the radiation dose.

It seems unlikely that the change in light absorption at 250 mμ reflects the radiation-induced inactivation of one of the sites essential for enzymic action since the loss in DNase I activity varied exponentially rather than linearly with the radiation dose and with the loss of tryptophan residue. It appears reasonable to postulate that the decrease in the concentration of five amino acids found after irradiation is sufficiently great to bring about a significant alteration in molecular structure leading to loss of enzymic activity of the DNase I molecules.

The change in amino acid composition after irradiation of DNase I differs qualitatively and quantitatively from that reported to occur in bovine albumin. The number of individual amino acid residues of DNase affected by irradiation is small compared with that of bovine albumin[1] and the decrease in concentration of the affected residues is generally smaller in DNase I than in bovine albumin[1].

Although a number of studies on the deamination of free amino acid by ionizing radiations have been reported[1, 10, 11], a comparison of the deaminative action of ioniz-

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ing radiations on the constitutive amino acids of DNase I with that on amino acid solutions seems unwarranted since the experimental conditions are not comparable. The ionic yield in terms of ammonia formation is a function of amino acid concentration, pH and radiation intensity and, in this respect, there seems no real basis for comparison. Furthermore, the respective state in which the amino acids are irradiated are obviously quite different.

SUMMARY

1. Significant changes in the amino acid composition of DNase I were observed after exposure of the enzyme to irradiation. Radiation-induced deamination appears to be the most important factor in the production of this change.

2. Exposure to large doses of ionizing radiation resulted in a significant change in the ultraviolet spectrum of DNase I. Although the absorption at 250 mμ increased linearly with radiation dose, the enzyme activity decreased exponentially.

3. These studies indicate that large doses of ionizing radiations sufficient to destroy the enzymic properties of this protein did not bring about an equally extensive change in the amino acid composition.

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THE ACTION OF CHYMOTRYPSIN ON N-ALKYL DERIVATIVES OF PHENYLALANINE ETHYL ESTER

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Studies by NEURATH *et al.*[1] have shown that chymotrypsin can act on ester linkages as well as on amide bonds, provided that the other specificity requirements are met. Thus, chymotrypsin readily hydrolyzes the ester group of such compounds as benzoyl L-tyrosine ethyl ester, benzoyl L-phenylalanine ethyl ester, acetyl L-tyrosine ethyl ester, etc. It was first found that the replacement of the "secondary peptide bond", such as the benzoyl amino or the acetyl amino group of the above compounds, by a

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