THE EFFECT OF DESOXYRIBONUCLEIC ACID ON THE RADIOSENSITIVITY OF α-CHYMOTRYPSIN IN AN ARTIFICIAL DNA–α-CHYMOTRYPSIN COMPLEX

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Because of the great biological importance of desoxyribonucleoproteins (DNP) and the comparative ease with which they are damaged by the action of ionizing radiation on the cell, in recent years the influence of ionizing radiation on these particular biological polymers has been investigated in vitro [3].

In these investigations injury to one of the components of the complex–desoxyribonucleic acid (DNA) –has been established. It has been shown by a comparative analysis of the radiosensitivity of DNA and DNP that the protein, entering into the composition of the DNP, increases the resistance of DNA to the action of radiation [8]. Until now, however, the possible influence of DNA on the radiosensitivity of the protein component of DNP has not been considered. The determination of this influence in the natural complex –the nucleoprotein– is impossible, for there is no method in existence which enables the specific biological activity of the histones and protamines, forming the main bulk of the protein of DNP, to be determined. This problem may, however, be solved by the use of artificial complexes of the enzyme protein with DNA. A comparison of the change in the activity of the enzymes in the free state and combined with DNA, under the influence of radiation, enables the influence of DNA on the radiosensitivity of the protein to be judged.

In the present research a complex of DNA with α-chymotrypsin was investigated. Damage to the protein after irradiation was estimated by the change in its proteolytic activity.

EXPERIMENTAL METHOD

DNA was obtained by the method of Mirsky and Pollister from the thymus and liver of cattle [10] and, by a slight modification of the method, from the pancreas [1], with subsequent deproteinization of the nucleoprotein by Sewag's method [12]; α-chymotrypsin was obtained by the method of Kunitz and Northrop [4]. The chymotrypsin was subjected to lyophilic drying or was preserved in 0.01N H_2SO_4 solution at a protein concentration of 4 mg/ml.

Complexes were obtained from the preparations of DNA and α-chymotrypsin; the reaction was carried out in a citrate buffer (pH 4.5) and in the same concentration of chymotrypsin as is present in the monomer state [2].

The DNA concentration was determined as phosphorus by the methods of Fiske-Subbarow or Spirin [5], and the nitrogen by Conway's method after mineralization with concentrated H_2SO_4 or by Nessler's micromethod. The molecular weight of the DNA was determined by the method of viscosimetry [6]. The proteolytic activity of the chymotrypsin and complexes was determined by Anson's method, by means of the amount of tyrosine liberated during incubation with substrate at pH = 7.6. After incubation for 20 minutes at 39° the protein was precipitated with 0.3N trichloroacetic acid solution and 2 ml of the filtrate was taken for determination of the tyrosine content with Folin's reagent in an alkaline medium. Irradiation was carried out with a RUM-7 x-ray apparatus at 60 kv, 20 ma, filter 0.5 mm Al and focus distance 75 mm.

EXPERIMENTAL RESULTS

Five preparations of complexes were obtained and their radiosensitivity studied. Their characteristics, and also those of the original DNA and α-chymotrypsin, are shown in Table 1.

We studied the action of various doses of radiation ranging from 50 to 50,000 r on α-chymotrypsin in the free state and combined with DNA. After the action of small doses no appreciable change was observed in the proteolytic activity of either the free enzyme or of the complex (Table 2). The variations in the results (± 5%) were within the limits of error of the method.

It may be seen from the results obtained by the action of moderate doses (Table 3) that the α-chymotrypsin in a complex with DNA was more sensitive to the action of radiation than α-chymotrypsin in the same concentration in the free state. The proteolytic activity

TABLE 1. Characteristics of the Original Components and the Resulting Complexes

Preparation No.	DNA			α-chymotrypsin	N/P of DNA–α-chymotrypsin complex
	source from which obtained	molecular weight	N/P		
1	Pancreas	$1.8 \cdot 10^6$	1.9	Series No. 1, preparation kept in 0.01N H_2SO_4 solution	9.9
2	The same	$1.8 \cdot 10^6$	1.9	The same	9.2
3	The same	$3.5 \cdot 10^6$	1.84	Series No. 2, preparation kept in 0.01N H_2SO_4 solution	8.0
4	Liver	$1.6 \cdot 10^6$	1.89	Series No. 3, after lyophilic drying	6.9
5	Thymus	$4.0 \cdot 10^6$	1.76	Series No. 3, after lyophilic drying	7.7

TABLE 2. Action of Small Doses of Radiation on Proteolytic Activity of α-Chymotrypsin in the Free State and as a Complex with DNA

Dose (in r)	Preparation No. 1			Preparation No. 2		
	concentration of chymotrypsin during irradiation (in mg/ ml)	proteolytic activity (as % of activity of unirradiated)		concentration of chymotrypsin during irradiation (in mg/ml)	proteolytic activity (as % of activity of unirradiated)	
		of free α-chymotrypsin	of α-chymotrypsin in complex		of free α-chymotrypsin	of α-chymotrypsin in complex
0	0.70	100	100	0.43	100	100
50	0.70	101	90	0.43	101	99
100	0.70	97	103	0.43	99	97
500	0.70	100	97	0.43	105	95

of the complex fell to 73% of the initial value when doses were used at which the activity of the free enzyme was not appreciably altered. With doses at which a fall in the activity of the free enzyme was observed, the activity of the complex fell to a much greater extent.

How may the greater degree of inactivation of chymotrypsin in the nucleoprotein complex be explained? It is presumable dependent on the disposition of the protein in the complex. Protein molecules, when they form complexes with DNA, are known to pass into a fully or partially opened state [7]. This shape of the protein probably facilitates inactivation during the action of radiation, by making the reactive centers more accessible to the action of free radicals formed by the radiolysis of water.

Inactivation may also be intensified as the result of preliminary partial denaturation, arising during the opening of the protein molecule in the process of complex formation. In this respect the findings in connection with alkaline phosphatase are of some interest, for its radiosensitivity increase sharply on its association with natural nucleoprotein [8], and the sensitivity of proteins to the action of radiation is also increased in a monomolecular layer, in which the protein is in the open state, as was observed in particular in experiments with pepsin [9]. It may therefore be considered that the cause of the greater radiosensitivity of protein associated with DNA is the open form in which the protein is present in the complex.

The fact that the radiosensitivity of an enzyme in the form of a complex is increased is interesting in connection with a further unsolved problem of radiobiology. Many enzymes are known to be much more resistant to irradiation in vitro than in vivo, although a considerable quantity of protective substances is present in the cell. The formation of a different group of complexes may become a method of increasing or weakening the radiosensitivity of individual biochemical components of the cell.

TABLE 3. Action of Moderate Doses of X-Rays on the Proteolytic Activity of α-Chymotrypsin in the Free State and as a Complex with DNA

Preparation No.	Concentration of α-chymotrypsin during irradiation (in mg/ml)	Dose (in r)	Proteolytic activity (as % of activity of unirradiated)	
			of free α-chymotrypsin	of α-chymotrypsin in complex with DNA
3	0.37	0	100	100
		10,000	98	91
		20,000	97	84
4	0.20	0	100	100
		10,000	98	91
		20,000	100	73
5	0.146	0	100	100
		25,000	65*	50
		50,000	42	26

*The radiosensitivity of chymotrypsin in this experiment was determined in a preparation stored for one year. It is not excluded that the radiosensitivity might be increased on account of partial denaturation during storage.

SUMMARY

The effect of DNA on the radiosensitivity of a protein included into the nucleoprotein complex was studied. Since biological activity, the change of which may conveniently serve as a criterion of protein disintegration, cannot yet be detected in the protein constituents of natural nucleoproteins, the authors used an artificial DNA–α-chymotrypsim complex for this investigation. It was found that after x-ray irradiation (10,000 and 50,000 r) of solutions of these complexes and of free α-chymotrypsim (control) the free enzyme is much more resistant to the action of the radiation than the same enzyme combined with DNA. The formation of different complexes may possibly be one of the factors which intensifies or reduces the radiosensitivity of the individual biological components of the cell.

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