Active Site of Deoxyribonuclease I.

III. Effects of Radiation on Deoxyribonuclease I in Dry State

by

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ABSTRACT

The mechanisms of inactivation of deoxyribonuclease by direct action of radiation have been studied. By testing kinetic properties of the enzyme and its tryptophan content, it seems likely that when the enzyme molecule absorbs energy from radiation, the energy is transferred to the 'active site' of the enzyme. This results in the degradation of one tryptophan residue, which, in turn, causes a loss of catalytic function of the enzyme. The differences between indirect and direct actions are also discussed.

INTRODUCTION

Previously, we have shown that deoxyribonuclease I irradiated with ionizing radiation in dilute aqueous solution is inactivated by radicals formed in the irradiated water, i.e. 'indirect action'. Inactivation of the enzyme resulting from the loss of its catalytic function is associated with the degradation of one specific tryptophan residue of the enzyme molecule (1, 2). When the enzyme is irradiated in the dry state, inactivation is caused by direct interaction of the ionizing radiation with the enzyme molecule, i.e. 'direct action' (3, 4). Since the mechanisms of enzyme inactivation by direct and indirect actions are different, the present investigation was initiated in order to determine how direct action modifies the active site of DNase molecules.

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80
METHODS AND MATERIALS

Deoxyribonuclease I (recrystallized once) purchased from Worthington Biochem. Co. was used without further purification. Five to eight mg. of dry enzyme was irradiated with gamma rays from a Cobalt 60 source in the presence of air. The dose rate ranged from 1400 to 2070 roentgens per minute, depending on the position of the sample with respect to the radiation source. The total radiation doses given ranged from $1 \times 10^6$ roentgens to $8 \times 10^6$ roentgens.

After irradiation, the irradiated deoxyribonuclease was weighted and dissolved in distilled water to make up solutions of 1 mg/ml. One portion of this solution was used to estimate tryptophan content (1). The kinetic properties of the irradiated enzyme (maximum velocity and Michaelis-Menten constant) were estimated by assaying the diluted enzyme solution at various concentrations of the substrate, deoxyribonucleic acid (2).

RESULTS

When the enzyme in the dry state was irradiated with various doses of gamma radiations and tested for enzyme activity at various substrate concentrations, the percent of enzyme activity remaining after irradiation is approximately an exponential function of the dose (Fig. 1) (3). By assuming an exponential relationship of inactivation to radiation dose, the calculated D$_{37}$ doses of the irradiated enzyme at various substrate concentration were found to decrease as the substrate concentration was increased (Fig. 2).

When the enzyme activity (v) was plotted against the substrate concentration (S) in the Lineweaver-Burk plot (Fig. 3), the value 1/V (the intercept of each slope with 1/v axis) increased as the radi-
ation dose was increased. Similarly, the value $1/K_M$ (the intercept of each slope with $1/S$ axis) also increased as the radiation dose increased. When $V$ (maximum velocity) and $K_M$ (Michaelis-Menten constant) of the enzyme irradiated with a given radiation dose were plotted against the radiation dose, Figure 1B was obtained. This shows that the maximum velocity ($V$) decreased almost exponentially as the radiation dose increased; this suggests a loss of catalytic function of the active site of the enzyme molecule. On the other hand, Michaelis-Menten constant ($K_M$) decreased as the radiation dose increased, indicating thereby an increased substrate-binding capacity of the enzyme molecule.

Since the previous studies in aqueous solution suggested that one specific tryptophan residue may be involved in the inactivation of the enzyme molecule, we have attempted to relate the number of tryptophan residues in the irradiated enzyme to the maximum velocity (Fig. 4). Extrapolation of the initial slope to zero enzyme activity indicated that one try-
ptophan residue was destroyed per one enzyme molecule inactivated.

DISCUSSION

The mechanism of inactivation of an enzyme irradiated in the dry state is believed to involve the absorption of energy by some parts of the protein molecule (5). The absorbed energy can migrate to other parts of the molecule where it may degrade certain amino acid residues and/or break chemical bonds responsible for secondary or tertiary structure of the protein molecule, i.e. hydrogen, S-S, hydrophobic bonds, etc. (5). The final events occurring in the irradiated molecule are undoubtedly of several types; however, the exponential nature of the inactivation curve suggests that one specific event is sufficient to inactivate the enzyme molecule.

Since kinetic studies indicate that enzymatic activity is associated with two functions, i.e. substrate-binding ability ($K_M$) and catalytic function ($V$), inactivation of the enzyme molecule could result from loss of one or both of these functions. In deoxyribonuclease I, it has been demonstrated that the inactivation of the enzyme is due solely to loss of its catalytic function. The substrate-binding ability, on the other hand, is increased after irradiation; in the presence of catalytic function, this would 'activate' the enzyme molecule.

The question as to the nature of the event in the irradiated molecule, which causes loss of catalytic activity, is of obvious interest. Since it is known that in many enzymes activity residues only in a part of a large protein molecule (i.e. the active site), the event is likely to involve destruction of all or part of the active site. The active site can be destroyed in two ways; first, by chemical change of certain amino acid residues or other chemical groups in the active site and secondly, by destruction of other chemical bonds (i.e. hydrogen, S-S, and hydrophobic bonds, etc.) which are not necessarily a part of the active site, but play a key role in maintaining the structural integrity of the active site.

Our previous studies with deoxyribonuclease (1, 2) indicate that one tryptophan residue is necessary for the biological activity of the active site. By estimating the tryptophan content of the enzyme irradiated in the dry state, a one-to-one relationship is observed between loss of one tryptophan residue and inactivation of one enzyme molecule. This suggests that loss of the enzymatic activity could be caused by destruction of one tryptophan residue in the active site.

The mechanism of direct action involves interaction of the enzyme molecule with the ionizing radiation, followed by 'energy transfer' within the molecule (3, 4). Indirect action, on the other hand, is the result of the reaction of the enzyme molecule with radicals formed in irradiated water. It seems likely that different chemical reactions occur in enzyme molecules as a result of these two mechanisms of inactivation. By comparing the present results with those obtained previously (1, 2) we note that in both cases the inactivation of deoxyribonuclease I results from the loss of its catalytic function. There is also a slight activation of the
enzyme in both cases, resulting from increased substrate-binding ability. The later phenomenon can be demonstrated only be use of low substrate concentrations in the enzyme assay system. The loss of catalytic function of deoxyribonuclease seems to be due to destruction of one specific tryptophan residue; however, there is one distinct and interesting difference in the inactivation caused by direct and indirect actions. Figure 4 shows that in direct action, one of the five tryptophan residues of deoxyribonuclease is destroyed much faster than the other four. In the case of indirect action, however, two tryptophan residues are more susceptible than the other three. This difference could be explained by the mechanisms responsible for tryptophan destruction in the two situations. Two tryptophan residues are probably located on the surface of the protein molecule, whereas three others are buried deep inside. In the indirect action, the exposed tryptophan residues are more susceptible to the active radicals than the other three inside the molecule. Similarly, when the N-bromosuccimide is added to the enzyme solution, the two exposed tryptophan residues are readily destroyed. In the direct action, however, energy is absorbed inside the protein molecule and transferred to locations able to trap such energy. It is conceivable that one tryptophan residue, if associated structurally with the active site, might be a better energy trap than the others and would therefore be more susceptible to the direct action of radiation.

SUMMARY

1. The kinetic properties of deoxyribonuclease I (maximum velocity and Michaelis-Menton constant) decreased upon irradiation in dry state in the presence of air.
2. One tryptophan residue was degraded per one molecule inactivated.
3. These results suggest that when deoxyribonuclease I was irradiated in dry state, some of the absorbed energy was transferred to the tryptophan residue of the active site, resulting in its degradation. This caused the enzyme molecule to lose its catalytic function.
4. The substrate-binding capacity of the irradiated enzyme was enhanced. This effect is demonstrable only when the irradiated enzyme is assayed in the presence of low substrate concentration.

REFERENCES