ENZYMATIC BREAKDOWN OF UV-IRRADIATED DNA BY THE EXTRACT FROM *Micrococcus lysodeicticus*

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In relation to the dark repair of ultraviolet light (UV)-irradiated bacteria, Elder and Beers (1965) recently obtained a fraction from extracts of *Micrococcus lysodeicticus* which could restore the transforming activity of UV-irradiated DNA from *Hemophilus influenzae*. Rörsch et al (1964) also obtained a fraction from the same bacteria which was active in the restoration of UV-irradiated replicative form ØX 174 DNA. These restorative processes were believed to be enzymatic and they seemed to shed light on the repair mechanism proposed by Setlow and Carrier (1964) and Boyce and Howard-Flanders (1964). In order to study the mechanism of this process we reinvestigated the extract from the same bacteria prepared with minor modifications according to Elder and Beers (1965) and we found that in the presence of Mg^{++}, UV- and heat-denatured DNA, but not native DNA, are enzymatically degraded by the extract. In this report the general features of the enzymatic breakdown of UV- and denatured DNA will be described. The results to be reported suggest that these degradative reactions are catalyzed by different enzymes.

Methods

Extracts were prepared from the dry powder of *M. lysodeicticus* commercially available from Miles Chemical Company, Clifton, New Jersey. The dried cells were

washed repeatedly with cold 0.01 M phosphate buffer, pH 7.2, until the washes were the pH of the buffer. Then it was suspended in the same buffer (10 ml/g dry powder) and incubated at 37°C for 1 hour. Following the incubation, 1.5 M NaCl was added to a final concentration of 0.01 M and lysozyme (Sigma Chemical Co., 2 mg/g dry powder), dissolved in 0.2 ml of the same buffer, was added and the mixture was re-incubated at 37°C with continuous stirring. After 17 to 20 min. when a hard curd had formed, the contents were chilled as quickly as possible by immersing in ice-water. The supernatant obtained by subsequent centrifugation for 30 min. at 10,000 X g was neutralized with Na_2HPO_4 and used as such. In contrast to the finding of Elder and Beers (1965), the supernatant obtained in this way was acidic and therefore Na_2HPO_4 should be added instead of KH_2PO_4. The content of the total protein determined by the phenol reagent of Folin (Lowry et al, 1951) was about 20 mg/ml

The enzymatic activity was determined by the amount of radioactivity liberated from the ^{32}P-labeled E.coli B/r DNA (Marmur, 1961) as perchloric acid-soluble material. The following reaction mixture was used: 0.1 M Tris-HCl buffer(pH 8.0), 0.1 ml; 2.5 X 10^{-2}M $MgSO_4$, 0.1 ml; ^{32}P-DNA, 0.1 ml (1 to 3 µg); extract, 0.1 ml.

Results

Inactivation of native- and UV-irradiated transforming DNA

Results are shown in Table 1. It can be seen that there is very little inactivation of native DNA during 3 hours of incubation while most of the surviving transforming activity of UV-irradiated DNA is nearly completely lost during the same period. The same tendency was recently reported by Reiter and Strauss (1965). It appeared therefore that the extract contained enzyme(s) specific for UV-irradiated DNA.

Degradation of native-, UV-irradiated,and heat-denatured DNA

The results obtained with transforming DNA was confirmed in studies of the degradation of labeled DNA. As can be seen in Fig. 1, UV-DNA is degraded more

Table I

The inactivation of native- and UV-irradiated transforming DNA of Diplococcus pneumoniae by the extract from M. lysodeicticus

UV-dose (ergs/mm^2)	number of transformants/ml	
	with extract	without extract
0	4×10^4	5×10^4
2.5×10^4	0	6×10^3
1.3×10^5	0	8×10^2

Solution of DNA in 0.15 M NaCl (4 μg/ml) was subjected to irradiation with UV. The composition of the reaction mixture is given in the text. The incubation was continued for 3 hours at 37°C and the mixture was diluted before assay of transforming activity.

rapidly than native DNA. It was also found that the extract contained a strong activity to degrade heat-denatured DNA. It became of interest to determine whether the degradation of UV- and heat-denatured DNA was catalyzed by different enzymes or by a single enzyme.

Effect of divalent cations

Since almost all the enzymes concerning degradation or synthesis of polynucleotides require divalent cation such as Mg^{++} or Mn^{++}, the effect of these cations as well as that of Ca^{++} was investigated. Results are shown in Fig. 2. It is seen that at 10^{-2}M both Mg^{++} and Mn^{++} are equally effective in the degradation of UV-DNA, whereas Mn^{++} at the same concentration inhibits the breakdown of denatured DNA. However at far lower concentrations, Mn^{++} acts as a stimulator for the latter reaction. In both reactions Ca^{++} acts as an inhibitor. It became evident in these experiments that the degradation of native DNA was stimulated weakly but significantly by Mn^{++}, but not by Mg^{++}.

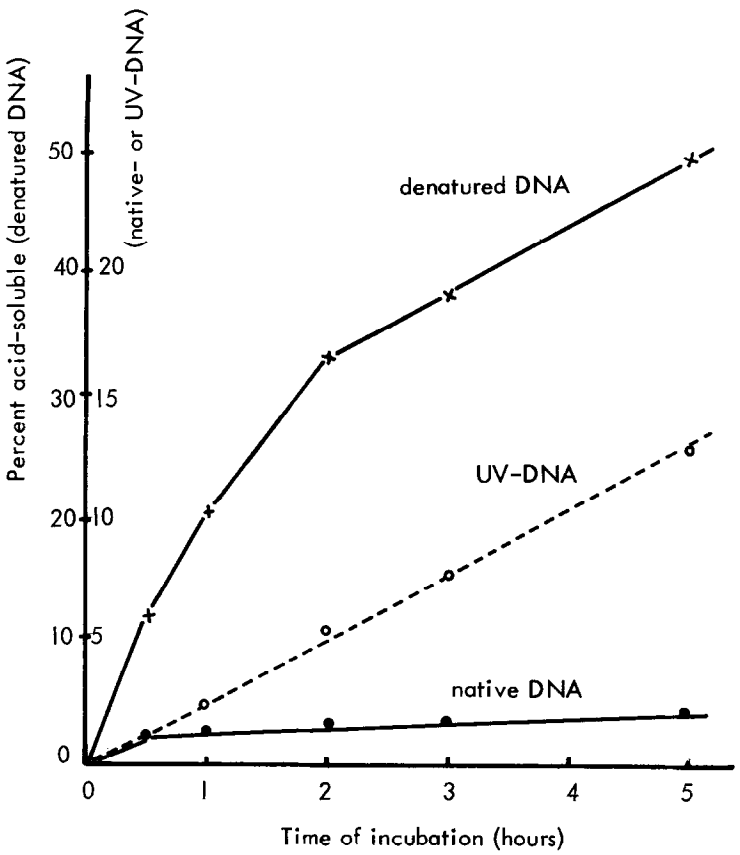

Fig.1 The degradation of native-, UV-irradiated, and heat-denatured DNA by the extract from M. lysodeicticus

The composition of the reaction mixture is described in the text. At various times, aliquots (0.4 ml) were cooled and mixed with carrier DNA (0.2 ml, 250 μg). To the mixture 0.2 ml of 6% perchloric acid was added and centrifuged in the cold. Aliquots of supernatants were dried and radioactivities were measured in a gas-flow GM-counter. Controls without extract were treated in the same way. For native- and UV-DNA the extract was used without dilution and for denatured DNA it was diluted ten-fold before use. UV-DNA was prepared by irradiating a solution of DNA (12 μg/ml) in 0.1 M Tris-HCl buffer, pH 8.0, for 10 min. (2.1×10^4 ergs/mm^2) with continuous stirring. Denatured DNA was prepared by heating the same solution of DNA in a boiling water bath for 15 min. followed by rapid cooling in ice-water (Marmur and Lane, 1960).

Effect of UV-dose

It is possible to imagine that a single enzyme attacks both UV- and denatured DNA, since according to Grossman et al (1961) UV-irradiation produces partial separation of the DNA duplex and the extent of denaturation is dependent upon the

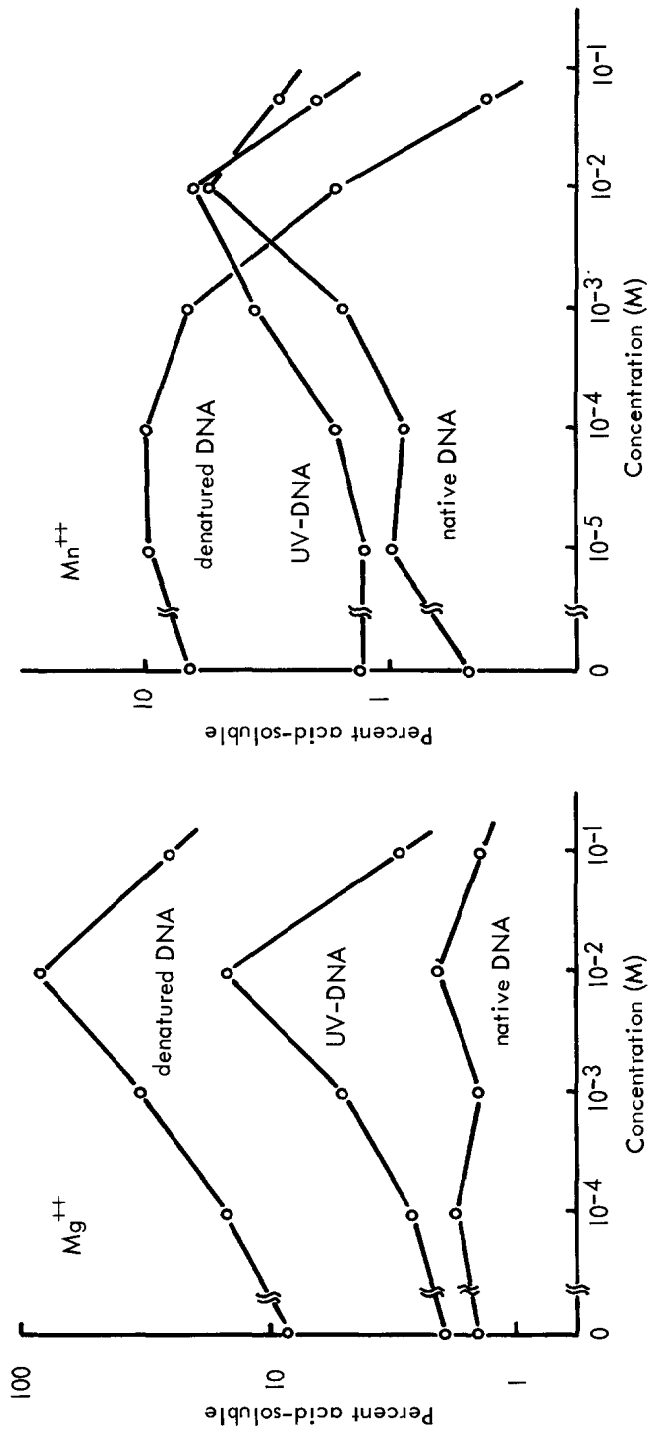

Fig. 2 Effect of divalent cations on the degradation of native-, UV-, and heat-denatured DNA by the extract from M. lysodeicticus

The method was outlined under Fig. 1. The radioactivity liberated in the acid-soluble fraction was measured after 3 hours of incubation.

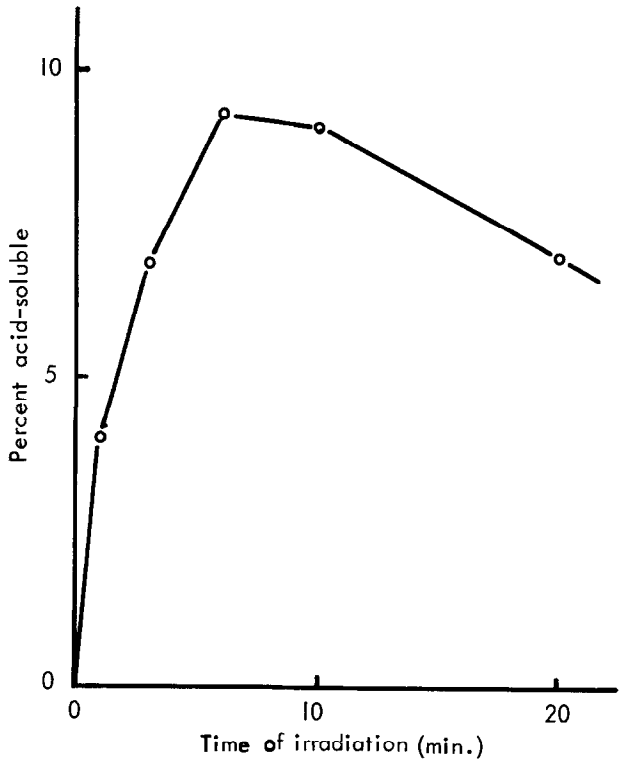

Fig. 3 Effect of UV-dose on the degradation of irradiated DNA by the extract from M. lysodeicticus
The reaction was arrested at 3 hours and the radioactivity in the acid-soluble fraction was measured in the same way as described under Fig. 1.

UV-dose up to very high doses. If this view is correct, the rate of breakdown of UV-DNA would be increased with increasing dose. Contrary to this assumption it was found (Fig. 3) that there was an optimal dose of UV for the degradation of DNA and that above this optimal dose of UV the degradation came to be inhibited a little, while the strand separation continues to increase (Grossman et al, 1961). Therefore the possibility that a single enzyme attacks both DNA seems to be quite unlikely.

Inhibition of the breakdown of UV-DNA by competing DNA

In order to check further the idea that the degradation of UV- and denatured DNA is catalyzed by different enzymes, experiments were performed in which various amounts of nonlabeled, denatured or native DNA were added to ^{32}P-labeled

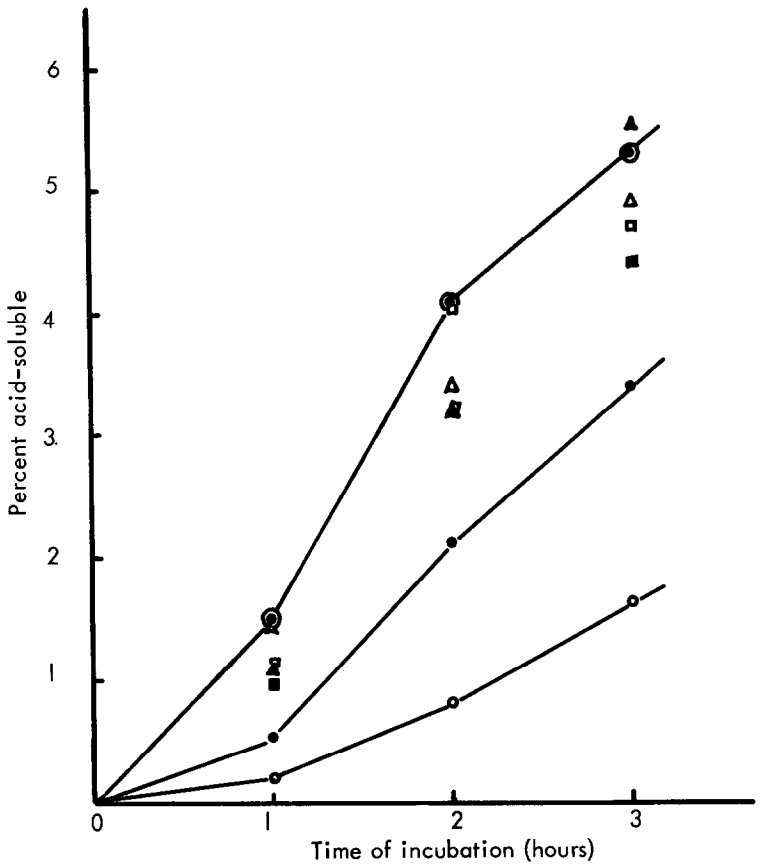

Fig. 4 The degradation of ^{32}P-labeled UV-DNA in the presence of nonlabeled UV-, native-, or heat-denatured DNA

In addition to the reaction mixture described in the text, 0.1 ml of nonlabeled DNA was added. The amount of the latter was taken to be equal or five-fold the amount of the labeled DNA. The reaction was stopped at 3 hours. —◉— , ^{32}P-DNA only (1 μg): triangles, nonlabeled native DNA; squares, nonlabeled denatured DNA; circles, nonlabeled UV-DNA: open symbols, 5 μg, closed symbols, 1 μg.

UV-DNA and the amount of acid-soluble radioactivity released was determined after three hours incubation with the extract. It is seen in Fig. 4 that degradation of ^{32}P-UV-DNA is not affected by the presence of five fold the amount of native- or denatured DNA, whereas it is strongly inhibited by the presence of equal amount of nonlabeled UV-DNA.

Discussion

The extract of M. lysodeicticus was shown to possess at least three kinds of

nuclease activity for DNA; very high activity for degradating denatured DNA and weak activities on UV- and native DNA. The following evidences strongly suggest that the breakdown of UV-DNA is catalyzed by a new enzyme which has a specific afinity for UV-DNA and that the coexisting enzymes which act on native- and denatured DNA are different ones. (a) The breakdown of labeled UV-DNA competed with nonlabeled UV-DNA but not with native- or denatured DNA. (b) The kinetics of the relationship between UV-dose and effectiveness with which the irradiated DNA is attacked is quite different from the relationship between strand separation and UV-dose observed by Grossman et al (1961). In so far as has been examined the nucleolytic activities in extracts of mutants of E. coli K12 and B, were weakened when substrate DNA was preirradiated with UV. According to Grossman et al (1961) UV-irradiation has no effect on the action of pancreatic DNase while the inhibition of labeled nucleotide release by E. coli phosphodiesterase is directly related to the extent of UV-irradiation. (c) Preliminary experiments showed that under certain conditions the activity for denatured DNA could be eluted faster from DEAE-cellulose column than the activity on UV-DNA.

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