A MUTANT OF MICROCOCCUS LYSODEIKTICUS DEFECTIVE IN A DEOXYRIBONUCLEASE ACTIVITY SPECIFIC FOR ULTRAVIOLET-IRRADIATED DNA

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Bacteria have an ability to repair ultraviolet light (UV)-induced damage to their DNA in the absence of visible light. It has been suggested that this process, called dark repair or host cell reactivation, involves the excision of a fragment of the nucleotides containing a thymine dimer from the DNA (Setlow and Carrier, 1964; Boyce and Howard-Flanders, 1964). The gap so formed is then filled by a process of repair replication (Pettijohn and Hanawalt, 1964).

In the preceding paper (Nakayama et al., 1967), we reported that an extract of Micrococcus lysodeikticus exhibited a deoxyribonuclease activity specific for UV-irradiated DNA (referred to as 'UV-DNase'). There arises a possibility that this activity is involved in the elimination of UV-induced damage in DNA. If this is the case, a mutant which lacks 'UV-DNase' must be sensitive to UV radiation, since the repair process cannot proceed in such a mutant.

The present paper describes the isolation and characterization of a M. lysodeikticus mutant which is defective in 'UV-DNase' activity. The mutant was found to have the same degree of UV-sensitivity and the same capacity to propagate UV-irradiated phage as the wild type has. These results suggest that the 'UV-DNase' activity is not concerned with dark

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repair.

MATERIALS AND METHODS

Most of the procedures have been described in the preceding paper (Nakayama et al., 1967).

A 'UV-DNase'-defective mutant was isolated as follows. A culture of M. lysodeikticus was treated with 1 mg/ml of N-methyl-N'-nitroso-N-nitroguanidine for 30 minutes in 0.1 M Tris-maleic buffer, pH 6.0. The treated cells were washed twice, resuspended in the original volume of nutrient broth, and shaken for 2 to 4 hours. The bacteria were plated on nutrient agar and incubated for 3 days at 37°. Each colony was inoculated into 0.5 ml of nutrient broth and shaken at 37° for 24 to 48 hours, depending upon its growth rate. Lysozyme (10 μg/ml) was added to each culture to lyze the cells. After the addition of UV-irradiated, ^{32}P-labeled DNA (0.1 to 0.5 μg in saline) and $MgCl_2$ (5 μmoles), the lysates were incubated at 37° for 60 minutes. The radioactivity in the acid-soluble fraction of each mixture was determined with a gas flow counter. Bacteria with low DNase activity were picked from the original plates as suspects and tested further, using both UV-irradiated and non-irradiated DNA as substrate.

UV-sensitive mutants were isolated among mitomycin C-sensitive colonies selected from mutagen-treated cultures by replica plating (Okubo and Romig, 1965).

RESULTS

A mutant defective in the 'UV-DNase': Among 1,500 colonies tested, one colony was isolated as a 'UV-DNase'-defective mutant (1312). Fig. 1 shows the breakdown of non-irradiated and irradiated DNA by extracts of the mutant and of the wild type. With the mutant extract the degradation of irradiated DNA proceeded in almost the same fashion as did that of non-irradiated DNA, whereas with the wild type extract irradiated DNA was degraded more rapidly

and more extensively than was non-irradiated DNA. As shown in Fig. 2, the

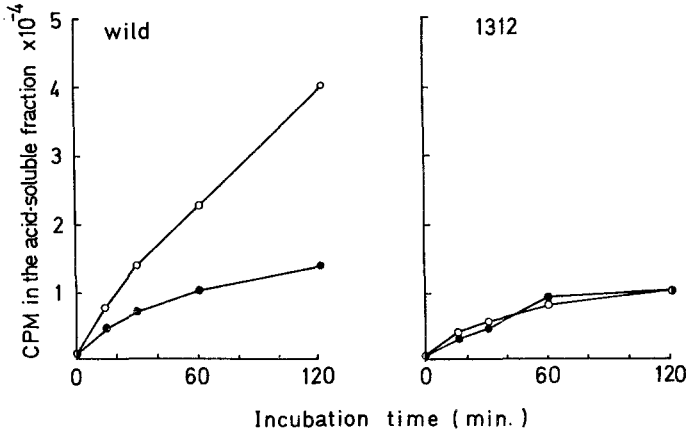

Fig. 1. The release of radioactivity into the acid-soluble fraction from DNA by extracts of M. lysodeikticus. The reaction mixture contained 20 μmoles of Tris-chloride buffer, pH 7.5, 5 μmoles of $MgCl_2$, 0.7 μg of labeled DNA (220,000 cpm) and extract (200 μg of protein) in 0.45 ml. Further details of the experimental procedures were given in the preceding paper (Nakayama et al., 1967).
—o— irradiated DNA ; —●— non-irradiated DNA

transforming activity of irradiated B. subtilis DNA was reduced when the DNA was incubated with the wild type extract, but not with the mutant extract.

It was shown in the preceding paper (Nakayama et al., 1967) that the 'UV-DNase' consists of two components, both of which are necessary for the selective breakdown of irradiated DNA. When the crude extract of 1312 was mixed with the fraction A derived from the wild type, 'UV-DNase' activity was observed (Fig. 3). The addition of fraction B from the wild type did not produce such an effect. When the mutant extract was subjected to chromatographic analysis as described in the preceding paper (Nakayama et al., 1967), no peak corresponding to fraction A was found, even in the presence of fraction B obtained from the wild type extract. Therefore, the mutant, 1312, seems to be defective in a component of fraction A.

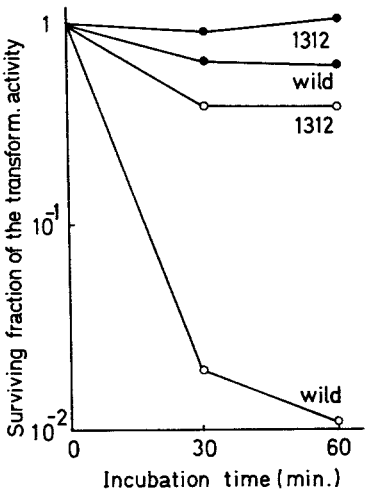

Fig. 2. The inactivation of the transforming activity of B. subtilis DNA by extracts of M. lysodeikticus. The experimental procedures were as described in the legend to Fig. 4 of the preceding paper (Nakayama et al., 1967). 0.2 ml of the extract (2.5 mg/ml protein) was added to the reaction mixture.
—○— irradiated DNA
—●— non-irradiated DNA

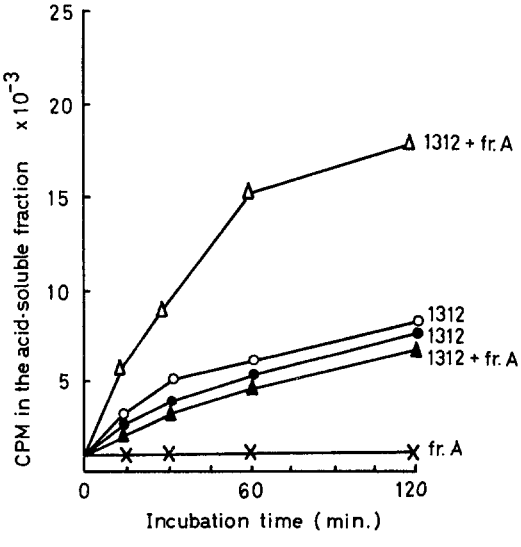

Fig. 3. Effect of fraction A of the wild type on the breakdown of irradiated DNA by the 1312 extract. The reaction procedures were the same as described in Fig. 1, except that the 1312 extract (50 μg of protein) was added per tube. As fraction A of the wild type, 0.1 ml aliquots of fraction #8 described in the preceding paper (Nakayama et al., 1967) were used.
—○— —△— irradiated DNA
—●— —▲— non-irradiated DNA
Incubation with fraction A alone gave almost the same result for either irradiated or non-irradiated DNA (—×—).

UV-sensitivity and host cell reactivation: To see whether 'UV-DNase' is involved in dark repair, the UV-sensitivity and the capacity to propagate

UV-irradiated phage (host cell reactivation) of the mutant were examined. For comparison, an UV-sensitive, host cell reactivation-negative (hcr$^-$) mutant of M. lysodeikticus was used. As shown in Fig. 4, 1312 has the same degree of UV-sensitivity and the same ability of host cell reactivation as the wild type has.

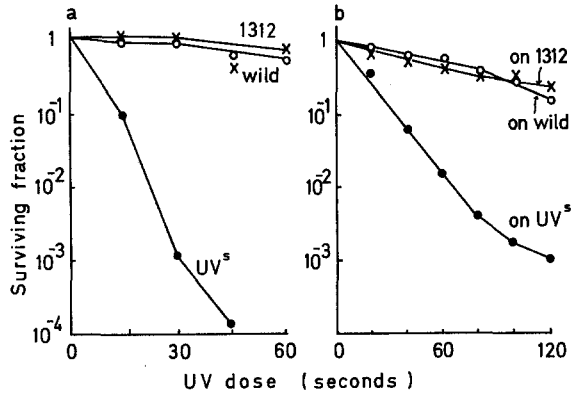

Fig. 4. (a) Survival of colony forming ability of M. lysodeikticus wild type, 1312, and of a UV-sensitive strain (UV^s) after UV-irradiation. Bacteria grown in nutrient broth were diluted 10 fold with 0.1 M Tris-chloride buffer, pH 7.2 and the dilution was irradiated at a distance of 50 cm from a 15 watt germicidal lamp.
(b) Host cell reactivation of N_6 phage. A phage suspension (1×10^8/ml in Tris buffer) was irradiated and plated using the indicator strains as shown in the figure.

DISCUSSION

In this paper, evidence was presented that a mutant of M. lysodeikticus, 1312, is defective in 'UV-DNase' activity. The mutant has the same level of UV-sensitivity and the same ability to reactivate UV-irradiated phage as the wild type has. Therefore, it may be concluded that 'UV-DNase' is not concerned with dark repair. This notion is supported by the fact that UV-sensitive and host cell reactivation-negative mutants so far tested (four mutants independently isolated) exhibit the normal level of 'UV-DNase' activity (unpublished results). However, our experiments could not completely eliminate possibilities such as the mutant having an altered 'UV-DNase' which might be

inactivated during the extraction procedures, or that the mutant might be so leaky in the activity of 'UV-DNase' that the repair process can take place *in situ*.

Strauss *et al.* (1966) have reported that breaks were produced in DNA containing UV-induced pyrimidine dimers by a *M. lysodeikticus* extract. It is necessary to investigate whether the activity they observed is due to 'UV-DNase' or not.

It may be pointed out that the mutant will be useful for enzymological study of the dark repair process. Irradiated transforming DNA of *B. subtilis* was inactivated by an extract of *M. lysodeikticus* wild type. When the mutant extract was used, almost all of the transforming activity was retained. Thus the mutant extract would be a good starting material for fractionation and purification of the enzymes responsible for each step of dark repair.

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