AN ENDONUCLEOLYTIC ACTIVITY SPECIFIC FOR ULTRAVIOLET-IRRADIATED DNA IN WILD TYPE AND MUTANT STRAINS OF MICROCOCCUS LYSODEIKTICUS

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An extract of Micrococcus lysodeikticus exhibits a deoxyribonuclease activity specific for ultraviolet light (UV)-irradiated DNA. First, Strauss (1962) reported an enzyme which inactivated the transforming activity of Bacillus subtilis DNA pre-exposed to UV, but not of untreated DNA. Recently, several investigators have reported the release of nucleotides from UV-irradiated DNA by extracts of M. lysodeikticus (Moriguchi and Suzuki, 1966; Carrier and Setlow, 1966; Nakayama et al., 1967). Furthermore, Strauss et al. (1966) showed that an extract from M. lysodeikticus induced breaks in UV-irradiated DNA.

We have shown in previous papers that two different fractions of M. lysodeikticus extract are required for the extensive release of nucleotides from UV-irradiated DNA in the presence of Mg^{++}, and that a mutant defective in the activity has been isolated (Nakayama et al., 1967; Okubo et al., 1967). This paper presents evidence that one of the fractions contains an endonuclease which acts specifically on UV-irradiated DNA, in the absence of Mg^{++}, and that the mutant is severely defective in this activity.

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MATERIALS AND METHODS

The wild type strain of M. lysodeikticus was provided by Dr. B. S. Strauss of the University of Chicago, and the mutant, 1312, was isolated from the wild type by Okubo et al. (1967).

Preparations of the extracts and enzyme fractions have been described in a previous paper (Nakayama et al., 1967). ^{14}C-labeled DNA was isolated by EDTA-SDS and phenol treatment of Escherichia coli C600 thy$^-$ which was grown in a 1 % casamino acid medium containing ^{14}C-thymine (2 μg/ml; 100 μc/mg). The DNA was irradiated in the cold with a 15 watt Toshiba germicidal lamp as follows; 80 sec. irradiation at a distance of 33 cm, or 30 min. irradiation at 23 cm. These correspond to approximately 2×10^3 and 1×10^5 ergs/mm^2, respectively, as estimated from the killing rate of λ phage.

Sucrose gradient centrifugations were carried out by layering a 0.1 ml sample containing 1 to 2 μg of labeled DNA onto a 5-20 % (w/v) linear sucrose gradient (4.7 ml) in either 0.15 M NaCl-0.015 M sodium citrate, pH 7.0 or 0.02 M K_3PO_4, pH 12.4, and centrifuging at 15° in the SW39 rotor of the Spinco L centrifuge. 10 drop fractions were collected from the bottom of each tube, and the radioactivity was determined in a liquid scintillation counter.

RESULTS

Effect of wild type and mutant extracts on UV-irradiated DNA: According to Strauss et al. (1966) an extract of M. lysodeikticus can induce breaks in UV-irradiated DNA. In order to see whether this activity is present in an extract from the mutant, 1312, which cannot selectively breakdown irradiated DNA, a comparison of wild type and mutant extracts was made using the procedure that Strauss et al. employed. As shown in Fig. 1, incubation of irradiated DNA with a wild type extract resulted in a marked decrease in the sedimentation rate of the DNA. In contrast, a crude extract of mutant 1312 did not noticeably change the sedimentation pattern of the irradiated DNA. The results obtained were qualitatively similar, whether the DNA preparation

had been exposed to a relatively low dose of UV, or to a high one, but more extensive degradation was observed with heavily irradiated DNA on treatment with the wild type extract.

Zone centrifugation in alkaline solution is a sensitive method for detecting

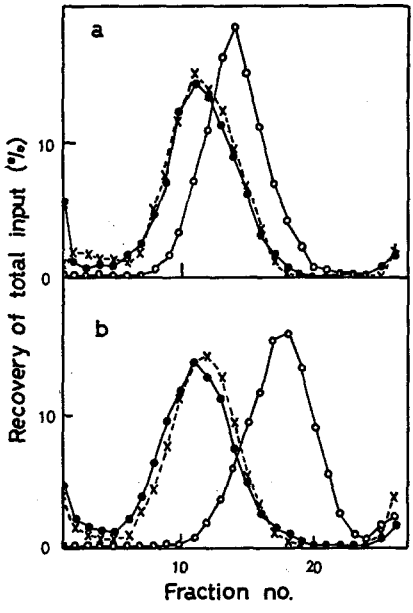

Fig. 1. Changes in the sedimentation patterns of UV-irradiated DNA by treatment with extracts of M. lysodeikticus. (a) DNA irradiated with a low dose of UV (ca. 2 x 10^3 ergs/mm^2). (b) DNA irradiated with a high dose of UV (ca. 1 x 10^5 ergs/mm^2). UV-irradiated DNA was treated with the wild type or mutant extract in the following reaction mixture: 0.2 ml of DNA (50 µg/ml; 3 x 10^6 cpm/mg), 0.1 ml of extract (10 mg protein/ml), 0.4 ml of 0.1 M EDTA-0.01 M tris-chloride, pH 7.8. After incubation at 37° in the dark for 30 min., an equal volume of phenol saturated with 0.15 M NaCl-0.015 M Na citrate was added, and the DNA was extracted by the slow rotation method. The phenol was removed by centrifugation and ether extraction. Conditions of centrifugation: 5-20 % sucrose gradient in 0.15 M NaCl-0.015 M Na citrate, pH 7.0, 4 hours at 30,000 rpm.
O—O with wild type extract
●—● with mutant 1312 extract
X— —X with water (untreated control)

single strand interruptions of the polynucleotide strands in DNA molecules (Vinograd et al., 1963). To investigate the strand interruptions more precisely, the same samples used in the experiment shown in Fig. 1 were analyzed by an alkaline sucrose density gradient centrifugation. The results, shown in Fig. 2, give the following information. (1) The breakdown of UV-irradiated DNA by the wild type extract is clearly demonstrated. Separation of the treated DNA from the untreated is more obvious in this analysis than in the neutral sucrose gradient analysis. (2) An extract of mutant 1312 is, to a slight extent, capable of decreasing the size of irradiated DNA. A few breaks which

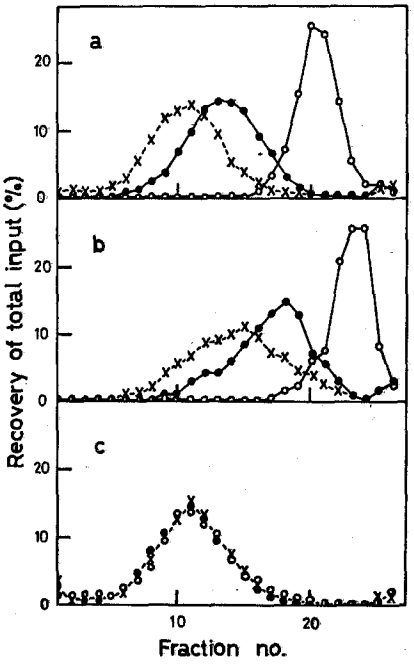

Fig. 2. Analysis by an alkaline zone centrifugation of extract-treated DNA. (a) DNA irradiated with a low dose of UV (ca. 2×10^3 ergs/mm^2). (b) DNA irradiated with a high dose of UV (ca. 1×10^5 ergs/mm^2). (c) non-irradiated DNA. Procedures are as described in Fig. 1. Conditions of centrifugation: 5-20 % sucrose gradient in 0.02 M K_3PO_4, pH 12.4, 4 hours at 35,000 rpm. O—O with wild type extract ; ●—● with mutant 1312 extract X- -X with water

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were not detected in neutral solution appear to be revealed by the strand separation in alkaline solution. (3) Heavily irradiated DNA (without treatment) moves more slowly with a somewhat spread boundary (Fig. 2b), indicating that a high dose of UV-irradiation itself induces breaks in DNA. Even with this kind of DNA, the difference between the effects of the two types of extract is evident. (4) Treatment of non-irradiated DNA with these extracts does not affect the size of the DNA (Fig. 2c).

It should be pointed out that all the experiments were performed in the presence of 0.06 M EDTA. Therefore the formation of breaks in irradiated DNA does not seem to require Mg^{++}.

Endonuclease activity in the chromatographed fractions: It has been shown in a previous paper (Nakayama et al., 1967) that two fractions, A and

B, are necessary for the extensive release of nucleotides from UV-irradiated DNA. In order to see whether one of these fractions contains the endonuclease activity, an experiment, the result of which is shown in Fig. 3, was performed.

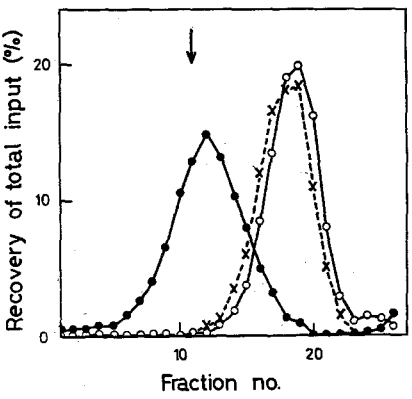

Fig. 3. Effect of fraction A and B of wild type extract on UV-irradiated DNA. Procedures are as described in Fig. 1. 0.1 ml each of chromatographed fraction A (0.1 mg protein/ml) and/or B (0.5 mg protein/ml) were used. UV dose given to DNA: ca. 2×10^3 ergs/mm^2. Conditions of centrifugation: 5-20 % sucrose gradient in 0.02 M K_3PO_4, pH 12.4, 4 hours at 35,000 rpm. An arrow indicates the position of a peak of untreated irradiated DNA.

o—o with fraction A
●—● with fraction B
X— —X with fraction A + B

It was found that fraction A alone can degrade irradiated DNA as efficiently as a crude extract of the wild type can, and fraction B has little effect on irradiated DNA. The size of the non-irradiated DNA did not change under treatment with either fraction or a mixture of the fractions. From these results it is evident that fraction A contains an endonuclease which acts specifically on UV-irradiated DNA in the absence of Mg^{++}.

DISCUSSION

We have confirmed the result reported by Strauss et al. (1966) that an extract from the wild type strain of M. lysodeikticus degrades DNA which contains UV-induced lesions in an endonucleolytic manner. Mg^{++} does not seem to be required for this reaction. An extract from mutant 1312 showed only slight activity in making breaks in irradiated DNA.

The relative molecular sizes of the DNAs treated with the two types of extract were calculated as follows. Burgi and Hershey (1963) found the rela-

tionship between the molecular weights (M) of linear DNA molecules and the sedimentation distances (D) in zone centrifugation to be $D_1/D_2 = (M_1/M_2)^k$, the value of k for alkaline-denatured DNA being given as 0.40 by Studier (1965). In the analysis shown in Fig. 2a, irradiated DNA preparations incubated with wild type extract, and with mutant extract, sedimented at the average rates of 0.37 and 0.83, respectively, of that of untreated DNA. From the equation the ratios of the molecular weights of extract-treated, and untreated DNA after denaturation are 0.083 for wild type extract and 0.63 for mutant extract.

The endonucleolytic activity was found in fraction A, which had been prepared by TEAE-cellulose chromatography from the wild type extract. Fraction B did not produce many breaks, nor was it necessary for the formation of breaks by fraction A. On the other hand, it was shown that both fractions, and also Mg^{++}, are required for the extensive release of nucleotides from irradiated DNA and that the mutant extract exhibits the nuclease activity characteristic of the wild type extract when fraction A from the wild type is supplied (Nakayama et al., 1967; Okubo et al., 1967). It seems, therefore, that an extensive degradation of UV-irradiated DNA occurs in the presence of Mg^{++} by a nuclease(s) in fraction B, after strand breaks near the UV lesions have been induced by the endonuclease, the activity of which does not require Mg^{++}, in fraction A.

It is supposed that the formation of a break near a pyrimidine dimer in one of the polynucleotide strands is the first step of dark repair (Setlow and Carrier, 1964; Boyce and Howard-Flanders, 1964). The endonuclease specific for irradiated DNA that we have observed could possibly be the enzyme responsible for this reaction. This is not likely to be the case since the mutant defective in endonuclease activity has the same UV-sensitivity as the wild type has (Okubo et al., 1967). However, a possibility remains that the weak endonuclease activity in the mutant, which we observed in an alkaline sucrose gradient centrifugation, might be sufficient for repair.

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