A DEOXYRIBONUCLEASE ACTIVITY SPECIFIC FOR ULTRAVIOLET-IRRADIATED DNA: A CHROMATOGRAPHIC ANALYSIS

Hiroaki Nakayama, Shunzo Okubo*, Mutsuo Sekiguchi and Yasuyuki Takagi

Department of Biochemistry, Kyushu University School of Medicine, Fukuoka, Japan

Received March 16, 1967

A crude extract of Micrococcus lysodeikticus has been found to recognize ultraviolet-induced modification of DNA: Strauss and his co-workers (1962, 1966) reported that an extract exhibited selective inactivation of transforming DNA pre-exposed to ultraviolet light (UV). This observation led us to look for a kind of DNase in the extract which was specifically active on UV-irradiated DNA. We have found that UV-irradiated DNA is more rapidly degraded by the extract than is non-irradiated DNA (Nakayama et al., 1966). Recently Moriguchi and Suzuki (1966), and Carrier and Setlow (1966) reported similar observations.

The present paper describes a chromatographic analysis of the extract, which demonstrates that at least two different components are involved in the selective degradation of UV-irradiated DNA.

MATERIALS AND METHODS

The strain of M. lysodeikticus used in the experiment was provided by Dr. B. S. Strauss of the University of Chicago. The cells were grown at 37° in nutrient broth with vigorous aeration. To prepare a crude extract, a cell suspension (5 % in wet weight per volume in 0.01 M potassium phosphate

---

* On leave from the Department of Genetics, Osaka University Medical School.

buffer, pH 7.0) was treated with 0.1 mg/ml of egg white lysozyme for 15 to 20 minutes and then sonicated for 10 minutes at 20 KC.

^{32}P-labeled DNA was extracted from E. coli B cells grown in Tris-casamino acid medium containing ^{32}P-orthophosphate. The extraction procedure included SDS-treatment, phenol extraction, precipitation by isopropanol, and RNase digestion, followed by a second phenol treatment and isopropanol precipitation. DNA (5 to 20 μg/ml in 0.15 M NaCl) was irradiated at a distance of 13 cm from a 15 watt germicidal lamp (Toshiba) for 20 minutes with continuous stirring.

The reaction mixture for the assay of DNase activity contained 20 μmoles of Tris-chloride buffer, pH 7.5, 5 μmoles of $MgCl_2$, 0.5 to 2 μg of labeled DNA and extract in 0.45 ml. After incubation at 37°, the radioactivity in the acid-soluble fraction of the reaction mixture was determined with a windowless gas flow counter.

The transforming activity of B. subtilis DNA was assayed by the method of Anagnostopoulos and Spizizen (1961).

RESULTS

The selective breakdown of UV-irradiated DNA: As shown in Fig. 1,

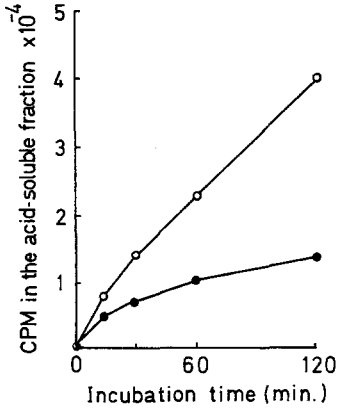

Fig. 1. The release of radioactivity into the acid-soluble fraction from non-irradiated and irradiated DNA by the extract of M. lysodeikticus. Experimental conditions were as described in the text. The reaction mixture contained extract (200 μg of protein) and 0.7 μg of DNA (220,000 cpm).
—●— non-irradiated DNA
—○— irradiated DNA

UV-irradiated DNA was more efficiently degraded than was non-irradiated DNA. These observations are in agreement with those reported by Moriguchi and Suzuki (1966).

Fractionation by column chromatography: The crude extract was fractionated by TEAE-cellulose column chromatography. When assayed with non-irradiated, irradiated, and heat-denatured DNA as substrate, the nuclease activity for

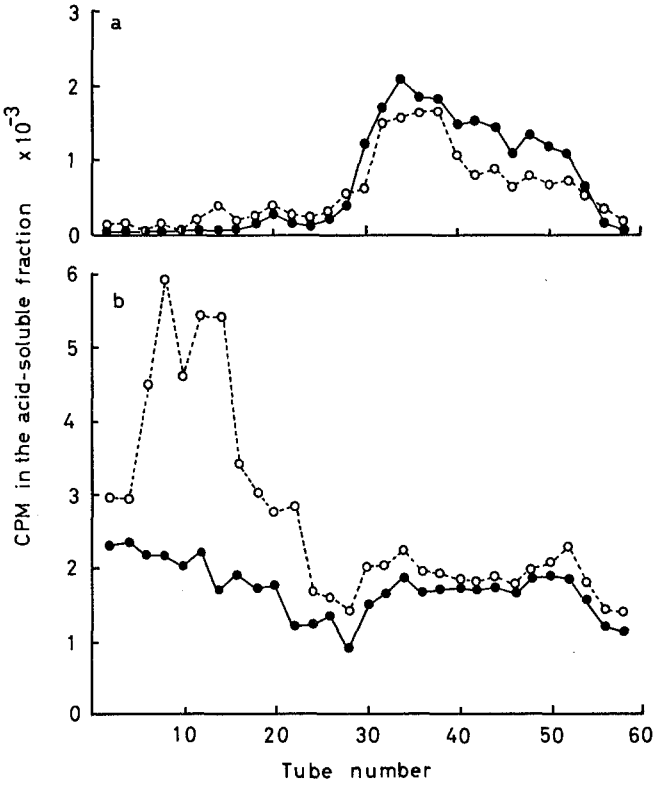

Fig. 2. TEAE-cellulose column chromatography of the extract. To the crude extract was added a one tenth volume of 5 % streptomycin sulfate and the resulting precipitate was spun off by centrifugation at 18,000 x g for 20 minutes. The supernatant was dialyzed against 0.01 M potassium phosphate buffer, pH 7.0-0.005 M 2-mercaptoethanol-0.001 M EDTA. The dialyzed extract containing 70 mg of protein was put on a TEAE-cellulose column (0.9 x 18 cm) equilibrated with the same buffer. After being washed, the column was eluted with a NaCl linear gradient (0 to 0.5 M in the same buffer, 110 ml of each). 4 ml fractions were collected and the enzymic activity of each fraction was assayed with non-irradiated, and irradiated DNA as substrate. The incubation time was 60 minutes. (a) 0.1 ml of each fraction. (b) 0.1 ml of each fraction + 0.1 ml of the peak fraction (fraction #35). 0.7 μg of DNA (70,000 cpm) was added to each reaction mixture.
—●— non-irradiated DNA ; ---○--- irradiated DNA

each DNA was found as a single peak at the same position. In contrast to the results with the crude extract, no selective degradation was observed with any of the chromatographed fractions. Non- and UV-irradiated DNA were degraded to almost the same extent by the peak fraction (Fig. 2a).

Since the activity on irradiated DNA seemed to be lost during the fractionation, the assay of each fraction was repeated in the presence of an aliquot of the peak fraction. In this case, the activity specific for irradiated DNA was clearly found at the beginning of the chromatogram (Fig. 2b). This fraction was referred to as A, and the fraction with non-specific nuclease activity, described above, as B. No peak was observed at the position of B in this chromatogram (Fig. 2b) probably due to the presence of large amounts of fraction B in each assay mixture. In Fig. 3, the time course of breakdown with these separate and combined fractions are shown.

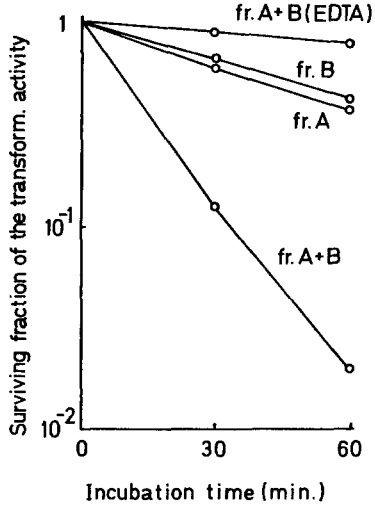

Fig. 3. The breakdown of non-irradiated and irradiated DNA by the TEAE fractions. The experimental conditions were as described in Materials and Methods. 0.1 ml aliquots of fractions A (#8) and B (#36) were used, and 0.5 μg (37,000 cpm) of DNA was added as substrate.
—●— non-irradiated DNA ; —○— irradiated DNA

The addition of fraction A to fraction B enhanced the breakdown of irradiated DNA but not of non-irradiated DNA. These data demonstrate that the combination

of fractions A and B is necessary for the selective breakdown of irradiated DNA.

Heat-denatured DNA was much more rapidly digested by fraction B than non-irradiated or irradiated DNA, as observed in the crude extract (Moriguchi and Suzuki, 1966). The addition of fraction A to fraction B did not enhance the breakdown of heat-denatured DNA.

The properties of fractions A and B: The active principles in both fractions were non-dialyzable and inactivated by treatment with phenol.

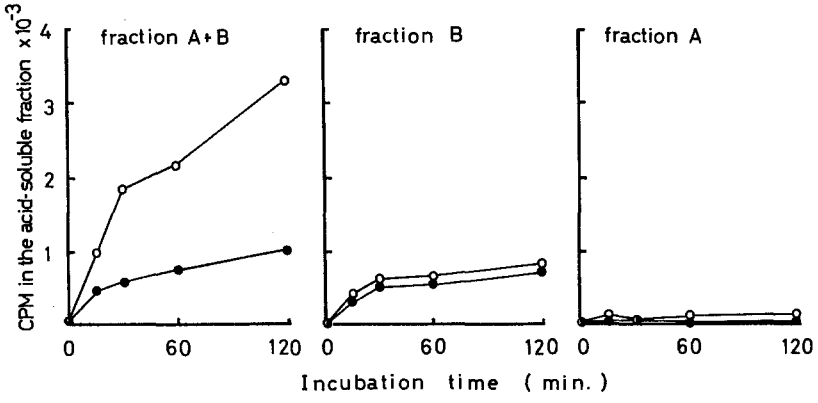

Fig. 4. The inactivation of B. subtilis transforming DNA by the TEAE fractions. Transforming DNA (50 μg/ml in saline-citrate) was irradiated for 3 minutes at a distance of 50 cm from a 15 watt germicidal lamp. The reaction mixture contained 0.2 ml of 0.2 M Tris-chloride buffer (pH 7.5), 0.1 ml of 0.1 M $MgCl_2$ or 0.25 M EDTA, 0.5 ml of DNA solution, and 0.2 ml of each of the fractions indicated in the figure. After incubation, 0.25 ml of 25 % SDS, 0.5 ml of 5 M sodium perchlorate, and 0.75 ml of saline-citrate were added. The mixture was shaken with an equal amount of chloroform-isoamylalcohol (24:1). After centrifugation, the upper layer was diluted 100-fold with saline-citrate and the dilution was assayed for its transforming activity. Curves for fr. A(EDTA) and fr. B(EDTA), not shown in the figure, were essentially the same as that for fr. A+B(EDTA). No appreciable loss of activity was observed with non-irradiated DNA, even in the presence of fractions A and B, and Mg^{2+}.

Heating at 100° for 10 minutes completely destroyed the activity of fraction B. The activity of fraction A appeared to be more heat-resistant; about 25 % of the activity remained after heating at 100° for 10 minutes.

The inactivation of transforming DNA: The results shown in Fig. 4 indicate that the selective inactivation of UV-irradiated transforming DNA also required the combination of fractions A and B. Mg^{2+} was necessary for this reaction, as was the case with the breakdown of DNA. The transforming activity of the non-irradiated DNA was not affected by treatment under the conditions used.

DISCUSSION

A cell-free extract of M. lysodeikticus exhibits a unique nuclease activity in degrading UV-irradiated DNA more efficiently than non-irradiated DNA. The present experiments reveal that this activity can be attributed to the combination of at least two components, the one, in fraction A exhibiting no nuclease activity as judged by the release of acid-soluble materials, and the other, in fraction B containing a non-specific nuclease activity(s), active on non-irradiated, irradiated, and denatured DNA. A preliminary experiment showed that sequential treatment with fraction A and then with B was also effective in the selective breakdown of irradiated DNA (unpublished results). Moreover, fraction A appears to have an endonucleolytic activity specific for the UV-induced lesions in DNA, as revealed by sucrose density gradient centrifugation (Shimada et al., 1967). A component in fraction B seems to be necessary for the release of acid-soluble materials from the irradiated DNA pre-attacked by fraction A. However, it is still unknown whether the non-specific nuclease(s) associated with fraction B is really involved in this process.

Heat-denatured DNA is also efficiently digested by the extract of M. lysodeikticus. This process, however, is different from that of the selective degradation of irradiated DNA, since the addition of fraction A, necessary for the latter process, does not enhance the breakdown of heat-denatured DNA by fraction B. Therefore, it is suggested that the component in fraction A is not active on the single stranded region, which might be produced by UV-irradiation as reported by Grossman et al. (1961).

The inactivation of transforming DNA exposed to UV also requires both components A and B, suggesting that the process is brought about by the deoxyribonuclease activity specific for irradiated DNA. This notion is supported by the fact that a crude extract of a mutant deficient in the nuclease activity does not destroy the transforming activity of irradiated DNA (Okubo et al., 1967).

ACKNOWLEDGEMENTS

This work was supported in part by grants from the Ministry of Education of Japan and from the National Institutes of Health, U. S. A. (12052). One of the authors (S. O.) is also indebted to the Japan Society for the Promotion of Sciences which enabled him to visit Kyushu University. Grateful acknowledgement is due to Mrs. T. Sata for her expert technical assistance.

REFERENCES

Anagnostopoulos, C., and Spizizen, J. (1961). J. Bacteriol., 81, 741.
Carrier, W. L., and Setlow, R. B. (1966). Biochim. Biophys. Acta, 129, 318.
Grossman, L., Stollar, D., and Herrington, K. (1961). J. Chim. Physique, 58, 1078.
Moriguchi, E., and Suzuki, K. (1966). Biochem. Biophys. Res. Comm., 24, 195.
Nakayama, H., Okubo, S., and Takagi, Y. (1966). J. Jap. Biochem. Soc., 38, 560 (an abstract in Japanese).
Okubo, S., Nakayama, H., Sekiguchi, M., and Takagi, Y. (1967). Biochem. Biophys. Res. Comm., following paper.
Shimada, K., et al., in preparation.
Strauss, B. S. (1962). Proc. Natl. Acad. Sci. U. S., 48, 1670.
Strauss, B. S., Searashi, T., and Robbins, M. (1966). Proc. Natl. Acad. Sci. U. S., 56, 932.