RESEARCH COMMUNICATIONS

A HUMAN ENDONUCLEASE ACTIVITY FOR GAMMA-IRRADIATED DNA

There is compelling evidence, in microorganisms, for DNA repair processes that contribute to recovery from radiation damage (Howard-Flanders, 1968). The excision repair system in particular has been extensively characterized at the molecular, enzymatic, and genetic levels (Setlow and Setlow, 1972). Although biochemical evidence for the excision system has been found in mammalian cells, its functional significance derives largely from analogy with microorganisms. The one exception is xeroderma pigmentosum (XP), a hereditary human skin disease in which cells are hypersensitive to UV light and deficient in the biochemical reactions of excision repair (Cleaver, 1968, 1971). So far none of the enzymes involved in mammalian excision repair have been positively identified. An endonuclease activity which acts on UV-irradiated DNA has recently been found in HeLa cells (Bacchetti et al., 1972; Brent, 1972) and in XP cells (Bacchetti et al., 1972). Since the function defective in XP cells appears to be an initial endonucleolytic incision at the site of UV-induced pyrimidine dimers (Cleaver, 1971), it follows that the human endonuclease activity assayed in vitro does not perform this function. This conclusion is reinforced by the observation that HeLa cell endonucleolytic activity persists after photoreactivation of UV-irradiated DNA (Bacchetti et al., 1972). On the premise that the observed endonuclease activity may act on a variety of lesions of a type distinct from UV dimers and that such lesions can be produced by modes of radiation other than UV, I examined gamma-irradiated DNA for susceptibility to endonuclease activity.

The enzyme extract was prepared by sonicating HeLa cells in 10 mM Tris-phosphate buffer, pH 8, and centrifuging at 100,000 *g* for 30 min. PM2 DNA, dissolved in 100 mM NaCl solution, 1 mM EDTA, 20 mM Tris-HCl, pH 7, at a concentration of about 100 μg per ml, was irradiated under air at room temperature with gamma rays from a ^{60}Co source at a dose rate of 330 rad per min. Endonuclease activity was assayed by incubating the PM2 DNA with the HeLa cell extract at pH 7 in the presence of 1 mM EDTA and subsequently separating the superhelical DNA substrate from the nicked DNA product by velocity sedimentation on alkaline sucrose gradients (Brent, 1972). Enzyme activity was calculated from the relative counts per minute under the respective peaks of radioactivity. All radioactivity on the gradients was recovered under the superhelical and nicked DNA peaks. The symmetrical shape of the nicked DNA peaks indicated the absence of extensive exonuclease action in these experiments.

In a typical experiment, 3,300 rad of gamma rays converted about 50% of the

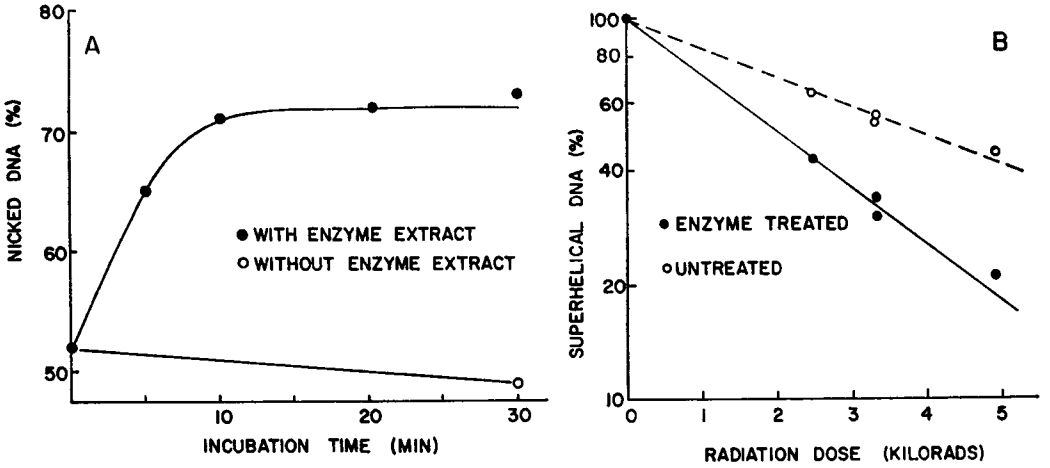

FIGURE 1 (A) Time-course of HeLa endonuclease action on PM2 DNA. The DNA was irradiated with 3,300 rad of gamma rays before incubation with or without HeLa cell extract. (B) Dose-response curves for production of radiation-induced single-strand breaks (○---○, D_{37} 5,800 rad) and of breaks produced by radiation plus postirradiation endonuclease action (●——●, D_{37} 3,000 rad).

superhelical DNA initially present to nicked circles. Upon incubation with HeLa cell extract there was a further time-dependent conversion of superhelical DNA to the nicked form, with a plateau reached by 10 min (Fig. 1 A). In the absence of cell extract, superhelical DNA was not converted to the nicked form. Nonirradiated DNA was not susceptible to the extract (Brent, 1972). This endonuclease activity was nondialyzable and heat labile.

Fig. 1 B shows DNA strand breakage as a function of radiation dose, with and without postirradiation incubation of the DNA with HeLa cell extract. The incubation period of 15 min allowed the endonuclease reaction to go to completion. The final extent of reaction represents the number of sites susceptible to the enzyme. Given that the molecular weight of PM2 DNA is 6×10^6 daltons (Espejo et al., 1969), one can calculate from the dose for 37% survival of superhelical DNA (Fig. 1 B) that 1 rad of gamma rays followed by maximum endonuclease reaction results in 55 breaks per 10^{12} daltons DNA, and that 1 rad of radiation with no subsequent endonuclease reaction produces 29 breaks per 10^{12} daltons DNA. The number of endonuclease-sensitive sites produced for each radiation-induced break is calculated from the ratio of these two values: (55:29) − 1 = 0.9. These enzyme-sensitive lesions may represent damaged DNA bases such as those selectively excised from gamma-irradiated *Micrococcus radiodurans* (Hariharan and Cerutti, 1972). The enzyme activity described here may be involved in the postirradiation strand breakage observed in L cells by Moss et al. (1971). Paterson and Setlow (1972) recently described an endonuclease activity in extracts of *M. luteus* that acts on gamma-irradiated DNA in *Escherichia coli* minicells. This endonuclease also appeared to act on UV-induced DNA damage and resembles the mammalian activity reported here.

Since the activity described in this report has been shown to act on UV-irradiated and now gamma-irradiated DNA, it probably has a broad spectrum of specificity. This possibility is currently being investigated with other DNA-damaging agents. The possibility that more than one enzyme is involved is being studied also. As yet there is no evidence that the observed endonucleolytic activity has any biological role in repair of radiation damage, and since these studies were with crude whole cell extracts, the activity could possibly represent lysosomal degradative enzymes.

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