SHORT COMMUNICATION

ABSENCE OF EXCISION REPAIR IN *NAEGLERIA GRUBERI*

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Protozoans are generally known to show a remarkable resistance to ionizing radiation. Although the mechanism for this resistance is not clear, possibilities such as polyploidy, high intracellular concentrations of SH compounds, and the presence of highly efficient DNA repair system have been proposed (Whitson 1972). *Naegleria gruberi*, a group of soil-water amoebae, is also resistant to X ray; irradiation with a 10^5 R does not appreciably change the count of viable cells (Fulton 1970). However, repair mechanisms for DNA damage induced by radiation have not been investigated on this species as the most cases of protozoa. This communication reports that this amoeba lacks the excision repair for DNA damage induced by UV which is known to work effectively in bacteria and some eucaryotes.

Methods used for the cultivation and the assay of viable cells are essentially the same as described by Fulton (1970). Suitable precautions were taken to minimize photoreactivation. The experiments were carried out under dim light. Figure 1 shows the UV survival curves of the vegetative phase cells of *N. gruberi* AB-2, which we isolated previously from soil. A pronounced sensitizing effect by caffeine post-treatment on the survival of AB-2 is evident. Since the postirradiation treatment with caffeine is generally believed to interfere with repair (Metzger 1964; Sauerbier 1964; Rauth 1967), this suggests that some repair mechanism sensitive to caffeine is operative in this amoeba. Acriflavin, which inhibits the excision repair (Feiner and Hill 1963), produced no UV sensitization at concentration up to $10 \mu\text{g/ml}$. The caffeine effect was also examined after incubation of the irradiated cells with or without bacteria as a food in liquid suspension at 33°C . Figure 2 shows that the irradiated cells become gradually immune to the sensitizing effect of caffeine during the incubation in the presence of a bacterial food. The caffeine-sensitive repair seems to be completed in 18 hr incubation. This repair did not work in the absence of a bacterial food supply.

We next examined the excision ability of the organism for UV-induced pyrimidine dimers. Amoeba cells were labeled by letting them feed overnight on *Escherichia coli* P3478, a thymine requiring strain which had been grown on nutrient agar plates containing $100 \mu\text{c}$ of ^3H -thymidine per plate. The amoebae were then fed unlabeled bacteria for 5 to 18 hrs to allow digestion of the labeled bacteria. Thymine dimer excision into acid-soluble fraction from DNA of UV-irradiated (2300 erg/mm^2) amoebae during the postirradiation incubation was measured according to the method of Sekiguchi *et al.*

Fig. 1. UV survival curves of *N. gruberi* AB-2 on ordinal nutrient agar plate (○) and caffeine-containing plate (●). Amoeba cells were grown on nutrient agar plates (PM plates) with bacteria. The bacteria were removed by differential centrifugation and the amoeba cells were resuspended at about 1×10^6 /ml in 0.002 M tris buffer (pH 7.4). Ultraviolet irradiation was carried out with a germicidal lamp at an incident intensity of 25 erg/mm²/sec. The cells were removed and assayed for viability by the technique of plating on PM plate with bacteria. The UV-irradiated cells were also plated on PM plate containing 500 μ g/ml of caffeine.

(1970). No excision was observed during the first 24 hr incubation.

Pyrimidine dimers in DNA can be precisely measured with an endonuclease selectively active to make incision at dimer-containing sites of DNA (Ganesan 1973; Paterson *et al.* 1973; Wilkins 1973). This method provides a simple and highly selective method for monitoring the disappearance of dimer-containing sites from DNA during the incubation of UV-irradiated cells. UV-irradiated, labeled amoebae were incubated in the presence of a bacterial food as described in the legend to Fig. 2. After 0 or 24 hr incubation, the amoeba cells were harvested and resuspended in saline-EDTA. DNA was prepared by lysing cells with 1% SDS followed by extraction with an equal volume of water-saturated phenol. The aqueous phase which contained DNA was dialyzed overnight at 4°C against the same buffer as used for assaying UV-endonuclease activity. Extraction of UV-endonuclease from *Micrococcus lysodeikticus*, enzymatic treatment of DNA and alkaline sucrose gradient analysis were done as previously reported (Nakayama and Okubo 1971). The result of the incubation of DNA isolated from UV-irradiated cells with the UV-endonuclease is shown in Fig. 3. The DNA extracted from cells

Fig. 2. Effect of caffeine as a function of the postirradiation incubation time. UV-irradiated (30 sec) amoeba cells in 0.002 M tris buffer were divided into two parts. To the one part bacteria suspended in 0.002 M tris buffer at a high concentration were added (left), and to the other the same volume of the buffer (right). The suspensions were incubated at 33°C with gentle shaking. At intervals aliquots were removed and assayed for survival on PM plates with (●) or without (○) caffeine.

after 24 hr postirradiation incubation in the presence of bacteria, which are supposed to have completed the caffeine-sensitive repair process from the data shown in Fig. 2, remained as sensitive to UV-endonuclease as DNA from non-incubated cells. In the control experiment, enzyme-treated DNA from unirradiated cells sedimented in alkaline sucrose gradients as fast as non-treated, unirradiated DNA. The lack of the disappearance of the nuclease-sensitive sites from DNA during the postirradiation incubation was also observed after a lower dose of 250 erg/mm².

The above lines of evidence suggest that the excision repair present in bacteria and various other organisms does not operate in *N. gruberi*. Freim and Deering (1970) reported that UV killing in a vegetative amoeboid growth phase of *Dictyostelium discoideum* is sensitive to caffeine, but not sensitive to acriflavin. Swinton and Hanawalt (1973) reported that *Chlamydomonas reinhardtii* lacks the excision mode of DNA repair. On the other hand, the excision of dimers in ciliate protozoa was reported by Sutherland *et al.* (1968) and Whitson *et al.* (1968). Thus, it appears that the phylum Protozoa is heterogenous with regard to the possession of excision repair system. The molecular nature of the caffeine-suppressible repair in *N. gruberi* remains to be unknown. Although it is likely to resemble the postreplication repair or tolerance repair mode documented in certain bacterial and eucaryotic systems (Kondo 1975), direct proof for this is still lacking.

Fig. 3. Sedimentation profiles in alkaline sucrose gradients of ^3H -labeled DNA extracted from UV-irradiated amoeba cells. DNA extraction was immediately after UV irradiation (upper) or after 24 hr incubation in the presence of bacteria (lower). DNA was treated (●) or not treated (○) with UV-endonuclease from *M. lysodeikticus* prior to sedimentation analysis.

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