RECOVERY FROM INHIBITION BY RADIATION OF TRANSCRIPTIONALLY CONTROLLED ENZYME INDUCTION

A possible probe for DNA repair

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1. Introduction

Most radiation effects like cell killing and mutagenesis are due primarily to DNA damage. As a result, DNA repair mechanisms play a major part in ameliorating such effects induced by ionizing and non-ionizing radiation [1–5]. Radiation-induced DNA lesions inhibit the template activity of DNA for DNA and RNA synthesis. The major effect of UV light photoproducts in DNA is in causing premature termination of RNA chains and release of RNA polymerase at the site of DNA photodamage [6]. We went one step further and studied the inhibition by DNA damage of transcriptionally controlled enzyme induction [7–9]. We now show that the transcriptionally-controlled induction of ornithine decarboxylase (ODC) can also serve as a probe to recovery mechanisms from radiation-induced DNA damage. It is suggested that the recovery we observe in Chinese hamster cells from inhibition of ODC induction may reflect repair of DNA damage.

2. Materials and methods

Chinese hamster V79 fibroblasts were grown attached to plastic petri dishes in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. The cells doubled in number in ~8–9 h at 37°C, in a humidified atmosphere containing 5% CO_2. For experiments, 3×10^4 cells were plated in 5 cm dishes. The cells reached confluency after 3 days and experiments were performed after 1–2 additional days when the cells were well in the plateau-phase (this can be judged by the yellow appearance of the growth medium) with $\sim 8 \times 10^6$ cells/dish.

At various times after induction the cells were rinsed with phosphate-buffered saline (PBS) and stored at −20°C. To each plate were added 2.5 ml assay buffer (50 μM ethylenediaminetetraacetic acid, 25 μM pyridoxal phosphate, 25 mM dithiothreitol, 25 mM Tris–HCl (pH 7.1)). The cells were removed from the surface using rubber policeman and subjected to 3 cycles of rapid freeze–thawing. The cell lysate was cleared by centrifugation at $2000 \times g$ for 10 min and used as such. The activity of ODC in cell extracts was determined by measuring the release of $^{14}CO_2$ from L-[1-^{14}C]ornithine (The Radiochemical Centre, Amersham) as in [10]. The activity in each cell extract was determined in duplicate and the average from duplicate plates were used for each datum point. Standard errors were 5–10% and are not shown.

3. Results

The effect of 75 krad γ radiation on ODC induction is shown in fig.1. When induction is triggered immediately after exposure there is ~50% inhibition of the activity of ODC which develops during up to 8 h. If induction is delayed for 1 h or 2 h after exposure, allowing the cells to recover from radiation damage, there is a progressive loss of the inhibition. Recovery appears to be complete in <2 h. Although 75 krad is a supralethal dose the cells remain viable for ≥ 8 h, as determined by trypan blue staining.

Fig.2 shows the effect of 5 J . m^{-2} of far UV light on ODC induction. At this dose, which also produces

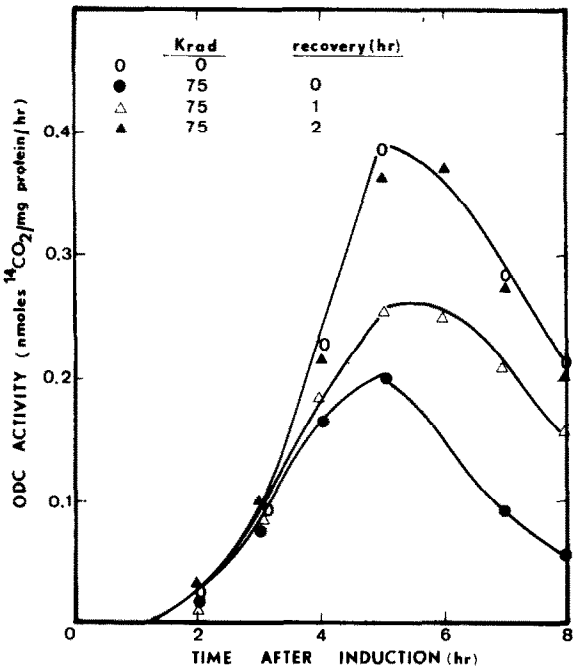

Fig.1. Effect of γ radiation. Chinese hamster cells in plateau-phase were exposed to 75 krad at room temperature in a Gammacell 200 (Atomic Energy of Canada Ltd.) equipped with a ^{60}Co source, at a dose rate of 1.2 krad/min. The growth medium was replaced with a fresh one at various times after exposure, as indicated, to induce ODC.

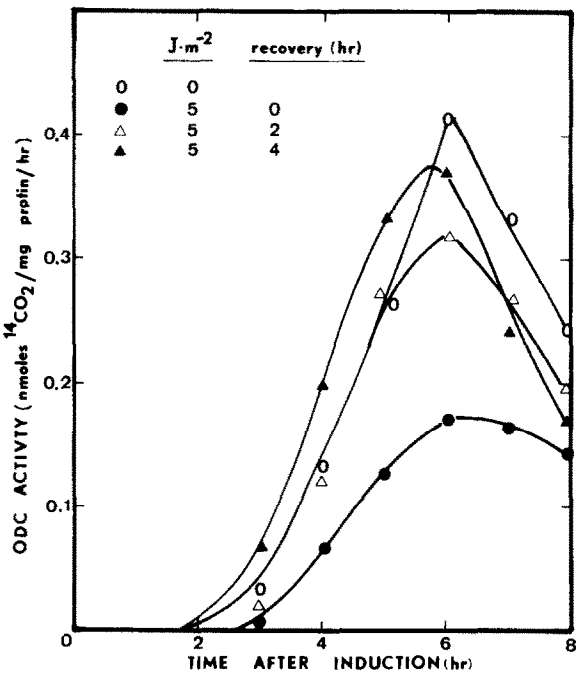

Fig.2. Effect of UV light. Chinese hamster cells in plateau-phase were exposed to 5 J . m^{-2} of far UV (254 nm) from a germicidal lamp (Philips TUV 15 W) at a flux of 0.5 J . m^{-2} . s^{-1}. The medium was replaced with PBS prior to exposure. After irradiation the PBS was removed and fresh DMEM added (no recovery). To study recovery the old DMEM was added back for 2 h or 4 h, as indicated, and then ODC was induced by the addition of fresh DMEM. ODC activity was determined at various times after induction.

~50% inhibition of ODC induction, most of the cells retain their proliferative ability. As in the case of γ radiation, a delay in induction allows the cells to recover from the inhibition. The recovery process is longer after exposure to UV light as ~4 h are required for complete recovery. It is interesting to note that, after 4 h recovery, ODC activity appears somewhat more rapidly than it normally does.

Recovery from inhibition of ODC induction takes place also after psoralen-plus-near UV (PUVA) treatment (fig.3). This recovery is even slower than that

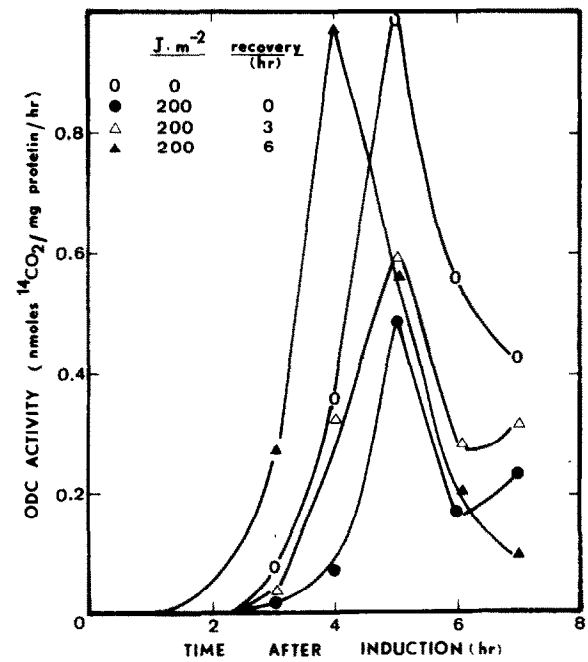

Fig.3. Effect of PUVA. Chinese hamster cells were exposed to 200 J . m^{-2} of near UV (300–400 nm) from two fluorescent black light lamps (F 15 T8-BLB, Sylvania Electric Products) held in a reflector at an incident flux of 10 J . m^{-2} . s^{-1}. Prior to irradiation the mediun was replaced with PBS containing 5 × 10^{-6} M, 4,5′,8-trimethylpsoralen (TMP). After exposure the TMP-containing PBS was removed and either fresh (no recovery) or the old DMEM was added back. The old medium was replaced with a fresh one after 3 h or 6 h, as indicated, for ODC induction. ODC activity was determined at various times after induction.

following exposure to far UV. It takes ~6 h after a dose of near UV (200 J . m^{-2}) that produces 50% inhibition of ODC induction. This dose kills 95% of the cells when survival is measured in terms of colony-forming ability (unpublished). The earlier development of ODC activity after 6 h recovery is more evident in the case of PUVA than following far UV light (fig.2).

4. Discussion

Taken together, the results clearly demonstrate the ability of plateau-phase Chinese hamster cells to recover from the inhibition of ODC induction by various kinds of radiation. There are, however, differences in the patterns of the observed recovery. Recovery appears to be fastest following γ-irradiation (2 h) and slowest after PUVA treatment (6 h) with far UV light intermediate (4 h). The rate of recovery from PUVA is very similar to the rate at which the cells remove TMP photoadducts from their DNA [11]. Ionizing radiation produces a whole spectrum of radioproducts in DNA. Those that are well studied, i.e., single-strand breaks [12] and 5,6-dihydroxy-dihydrothymine [13] are repaired in <1 h. This is faster than the recovery from inhibition of ODC induction and may suggest the existence of additional type of damage that is repaired more slowly.

Recovery from inhibition by far UV light is more difficult to explain in terms of repair of DNA damage. This is because Chinese hamster cells excise UV-induced pyrimidine dimers very inefficiently, ~30% in 24 h [14]. However, since these cells are not unusually UV-sensitive, they must possess some other mechanism to overcome the damage remaining in their DNA. Our present knowledge does not allow any precise interpretation of the UV data.

Since it takes ~1 h to transcribe most of the RNA species required for the development of ODC activity after induction [9], some recovery could take place even after the old medium is replaced by a fresh one immediately after irradiation. This is true especially in the case of ionizing radiation where recovery is quite rapid. This possibility can be tested only by comparing the dose response for inhibition in normal cells to that of cells deficient in DNA repair (e.g., *Xeroderma pigmentosum* cells' response to far UV light).

In conclusion, ionizing and non-ionizing radiations inhibit the transcriptionally-controlled induction of ODC in plateau-phase Chinese hamster cells. The cells are able to recover from this inhibition at a rate which is typical for each kind of radiation. It is suggested that the recovery process reflects repair of radiation damage in DNA and that it could serve as a probe to study the ability of the cell to perform repair. Other assays of DNA repair measure specific steps in this complex process. Since the rate-limiting step is usually not defined, the biological significance of such measurements with regard to the capacity of the cells to recover from radiation damage is not straightforward. The ODC system could serve as a biochemical assay which measures the end result of the repair process, i.e., the ability of the cells to transmit intact message for the synthesis of an active enzyme molecule, following restoration of the DNA template, during the first few hours following the production of damage in DNA.

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