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The involvement of indirect effects in cell-killing and DNA double-strand breakage in γ-irradiated *Escherichia coli* K-12

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1. Introduction

When cells are irradiated with X- or γ-radiation, radiochemical damage is deposited in the DNA as a result of direct and indirect processes. The direct process is one in which energy is deposited in the target molecule by the ionization itself, possibly resulting in inactivation of that molecule. The indirect process, however, is one in which inactivation of the sensitive target molecule is mediated by products of the interaction of the radiation with other molecular components in the milieu of the target (see Okada 1970). Although it is known that it is the OH· radical which is responsible for most of the indirect contribution to the inactivation of *E. coli* (Johansen and Howard-Flanders 1965), it is not known what lesion the radical produces that results in cell death. We sought to ask how cell-killing and the production of a known irrepairable and presumably lethal lesion, DNA double-strand breaks, change when cells are irradiated in the presence of glycerol, a known scavenger of OH· radicals.

2. Materials and methods

Escherichia coli K-12 W3110 cells were grown in minimal medium to mid-log phase ($\sim 2 \times 10^8$ cells/ml), as previously described (Bonura, Town, Smith and Kaplan 1975). Cells to be used for DNA breakage experiments were grown in the presence of 200 μCi/ml of ^{3}H-thymine (New England Nuclear, > 15 Ci/mmol) to label the DNA fully. The cells were collected by filtration onto membrane filters (0·45 μm pore size; Millipore Corporation) and resuspended in DTM buffer (minimal medium without the organic supplements) at a density of $\sim 2 \times 10^8$ per ml for survival studies or at $\sim 3 \times 10^7$ per ml for studies on DNA double-strand breakage. The cells were either irradiated in DTM only or in DTM plus 1 M glycerol, a concentration which provides nearly maximum protection (data not shown). Irradiations were carried out with ^{137}Cs γ-rays using a Mark I irradiator (J. L. Shepard and Associates) at a dose-rate of 5·7 krad/min as determined by ferrous sulphate dosimetry. The cell suspensions in a water-jacketed glass irradiation vessel were equilibrated with air by bubbling for 5 min before and during irradiation. The neutral sucrose gradient technique has been described in detail elsewhere (Bonura and Smith 1975, Bonura *et al.* 1975).

Figure 1 shows survival curves for wild-type *E. coli* K-12 cells irradiated at room temperature (23°C) in the presence and absence of 1 M glycerol. The D_0 values taken from the exponential portion of the survival curves are $7{\cdot}77 \pm 0{\cdot}24$ krad and $3{\cdot}14 \pm 0{\cdot}12$ krad for irradiations in the presence and absence of 1 M

glycerol, respectively. The degree of protection by glycerol is $2{\cdot}47 \pm 0{\cdot}12$, in agreement with results obtained by other investigators (Sanner and Pihl 1969, Donta and Freifelder 1970). The production of DNA double-strand breaks is shown in figure 2, plotted as the reciprocal number-average molecular weight as a function of dose. Irradiation in the presence of glycerol reduced the yield of DNA double-strand breaks by a factor of $2{\cdot}42 \pm 0{\cdot}37$ based on the slopes of the regressed lines shown in figure 2.

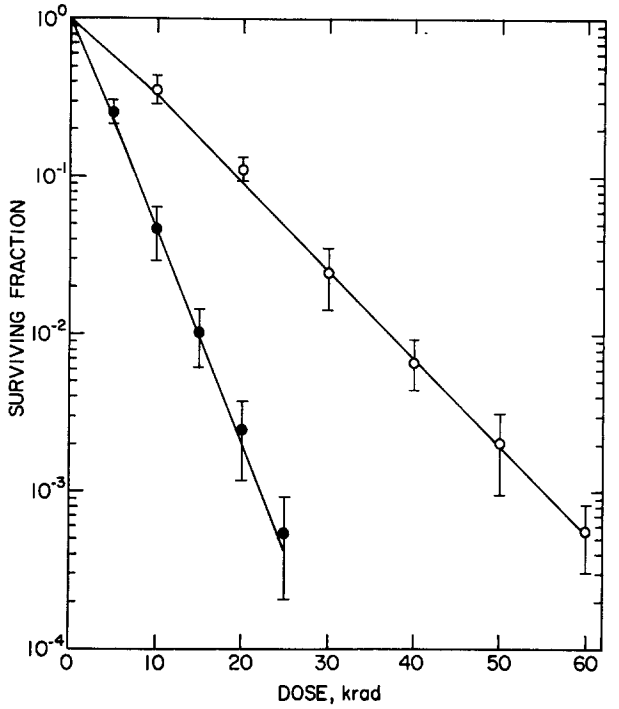

Figure 1. Survival curves for *Escherichia coli* K-12 W3110 γ-irradiated at ~23°C under aerobic conditions in DTM buffer with (○) or without (●) 1 M glycerol. After irradiation cells were diluted in 0·067 M phosphate buffer pH 7·0, plated on minimal medium agar and incubated at 37°C for 2–3 days to allow for colony formation. The data are the average of four experiments. The curves were fitted by a least-squares regression analysis excluding the data for unirradiated cells.

We have previously shown a close correlation between the X-ray dose required to introduce a DNA double-strand break into the *E. coli* genome and the D_0 dose based upon survival both under aerobic and anoxic conditions (Bonura *et al.* 1975). The dose required to produce a DNA double-strand break in the *E. coli* genome ($2{\cdot}8 \times 10^9$ daltons) as calculated from the regression lines in figure 2 is $10{\cdot}0 \pm 1{\cdot}3$ krad in the presence of glycerol and $4{\cdot}14 \pm 0{\cdot}33$ krad in the absence of glycerol. These values compare favourably with the D_0 values of 7·77 and 3·14 krad for survival in the presence and absence of glycerol, respectively.

Our data show that glycerol decreases the yield of DNA double-strand breaks and protects *E. coli* in terms of survival to an equivalent degree. Because DNA double-strand breaks are introduced by a dose which closely corresponds to that required to introduce a lethal event, it seems reasonable that the protective

effect of irradiation in the presence of glycerol may be a function of its inhibition of the formation of DNA double-strand breaks. Using bacteriophage λ, however, Donta and Freifelder (1970) found that the protection afforded by glycerol against the lethal effects of X-irradiation was due both to a decrease in yield of DNA double-strand breaks and base damage.

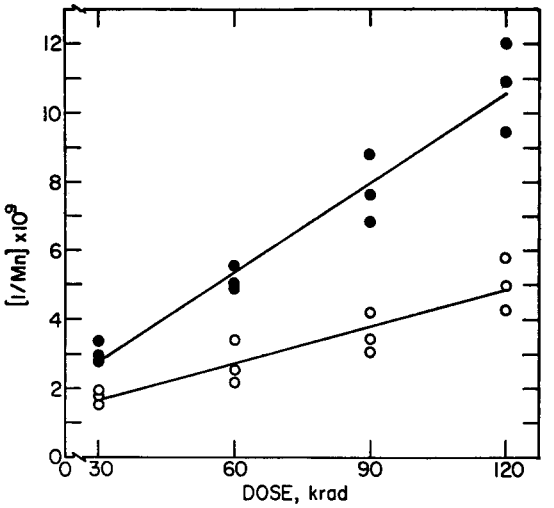

Figure 2. The production of DNA double-strand breaks after γ-irradiation of *E. coli* K-12 cells at 23°C under aerobic conditions in buffer with (○) or without (●) 1 M glycerol. The number of DNA double-strand breaks is proportional to the reciprocal number-average molecular weight (1/M*n*). The curves were fitted by a least-squares regression analysis excluding the data for unirradiated cells.

Our data also show that a significant number of DNA double-strand breaks arise by indirect action, probably due to attack by OH· radicals. This is especially surprising in view of the linear kinetics by which DNA double-strand breaks are produced (figure 2 and Frey and Hagen 1975), since DNA double-strand breaks arising from two independent events should follow two-hit kinetics. A reasonable explanation for the linear dose-response may be that a primary radiation-induced single-strand break is produced on one strand along with a spur of OH· radicals which are free to attack the opposite strand to produce an opposed single-strand break. The result would be that DNA double-strand breaks would appear to be produced by a one-hit process (i.e., to increase linearly). The diffusion distance of the OH· radical is approximately 2·5 nm (Sanner and Pihl 1969, Hutchinson 1961), making such a mechanism possible. Furthermore, there are indications that radical attack in the vicinity of a single-strand break may be enhanced owing to the loss of base pairing in such a region (Swinehart, Linn and Cerutti 1974, Frey and Hagen 1975).

Although the production of other DNA lesions, such as base damage (Donta and Freifelder 1970, Roti Roti and Cerutti 1974) and single-strand breaks (Donta and Freifelder 1970, Roots and Okada 1972), is also inhibited in bacteriophage and mammalian cells by the presence of glycerol during irradiation, it seems more reasonable to ascribe protection in terms of survival in *E. coli* to an inhibition of DNA double-strand breakage. This seems justified since base

damage and single-strand breaks appear to be repairable lesions in this strain of *E. coli*, while DNA double-strand breaks are not.

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