

Yield of X-ray-induced DNA single-strand breaks in niacin-starved *E. coli* K-12

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(Received 13 July 1972; accepted 21 July 1972)

1. Introduction

We have recently presented evidence for the existence of an ultra-fast repair process in *E. coli* K-12, which acts mainly on DNA single-strand breaks produced by x-irradiation under anoxic conditions (Town, Smith and Kaplan 1972). This process is not inhibited at 0°C under normal conditions, and on its completion leaves about three times as many DNA strand breaks unrepaired in aerobically-irradiated cells as in cells irradiated under anoxic conditions. Since it is thought that DNA (polynucleotide) ligase is required in the final rejoining step in this process, we examined the yield of x-ray-induced single-strand breaks in cells that had been starved of niacin. Such starvation depletes the pools of nicotinamide adenine dinucleotide (NAD) in niacin-requiring cells, and causes an inhibition of DNA ligase, for which NAD is a required co-factor (Nozawa and Mizuno 1969).

Our results show that niacin starvation of wild-type cells caused a 1.9-fold increase in the number of breaks observed immediately after anoxic irradiation but had a much smaller effect (1.2-fold increase) on the yield of breaks under aerobic conditions, when compared with the yields of x-ray-induced DNA single-strand breaks in *polA1* cells (lacking DNA polymerase I) irradiated under similar conditions.

2. Materials and methods

The niacin-requiring derivative of *E. coli* K-12 (W3110) *thy, nad* isolated and described by Nozawa and Mizuno (1969) was used. It was further characterized in this laboratory as requiring threonine and leucine, so that it could be grown in the absence of casein hydrolysate.

Cells were grown at 37°C with aeration to log phase ($1-2 \times 10^8$ cells/ml) in a glucose salts medium (Ganesan and Smith 1968) supplemented with thymine at 2 µg/ml (10 µg/ml in overnight cultures and in plates), threonine and leucine to 10^{-3} M and niacinamide (which can substitute for niacin) at 0.1 µg/ml. Cells were starved in the same medium by the omission of niacinamide. Transfer was accomplished by Millipore filtration and three washings in warm niacinamide-free medium.

To determine cell viability, cells were diluted in phosphate buffered saline (PBS), pH 7.35 (NaCl 8.0 g, KCl 0.2 g, Na_2HPO_4 1.15 g, KH_2PO_4 0.2 g, H_2O

to 1.0 litre) and spread on plates containing the above growth medium solidified with 0.9 per cent Oxoid agar-agar No. 3.

For irradiation, cells in exponential growth (at $1-2 \times 10^8$ cells/ml) were collected by Millipore filtration and resuspended in PBS at room temperature.

To label the DNA with radioactivity for sedimentation studies, cells were grown for three generations in the medium described above, except that the thymine concentration was reduced to $1.34 \mu\text{g/ml}$ and contained $50 \mu\text{Ci/ml}$ ^3H -thymine (New England Nuclear; 15–20 Ci/mmol). The labelling took place before the beginning of the niacin starvation period. Details of the irradiation (using 50 kVp x-rays), sedimentation procedures, and analysis of results have been described previously (Town *et al.* 1972).

3. Results

During niacin starvation, cells undergo only 1–2 divisions and show a progressive increase in x-ray sensitivity (figure 1). The same response is seen whether cells are plated on minimal or complex medium. Sensitivity reaches a maximum after 7–8 hours. Starvation overnight (16–18 hours) gives a slightly higher survival after irradiation relative to the unirradiated cells, which is attributable to some loss of viability in the unirradiated cells.

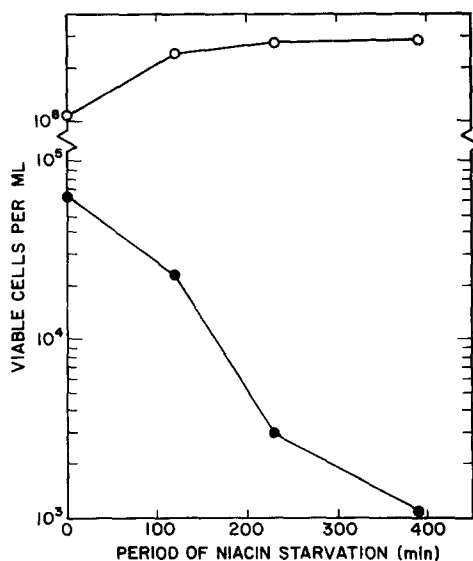


Figure 1. Effect of niacin starvation on growth and radiosensitivity of *E. coli* K-12. Cells growing exponentially in minimal medium were transferred to medium without niacin and incubation continued without niacin. At intervals, cells were suspended in PBS, irradiated, and plated for colony formation. ○, unirradiated; ●, irradiated (20 krad, air equilibrium).

To investigate the possibility that a ligase reaction (involving NAD as a co-factor) is responsible for the differential yield of single-strand breaks in cells irradiated in the presence or absence of oxygen, the yield of single-strand breaks was measured in cells that had been starved of niacin for 18 hours before irradiation. To inhibit polymerase repair, the experiments were carried out at 0°C in the presence of 2×10^{-3} M NaCN, and the ^3H -labelled cells were lysed

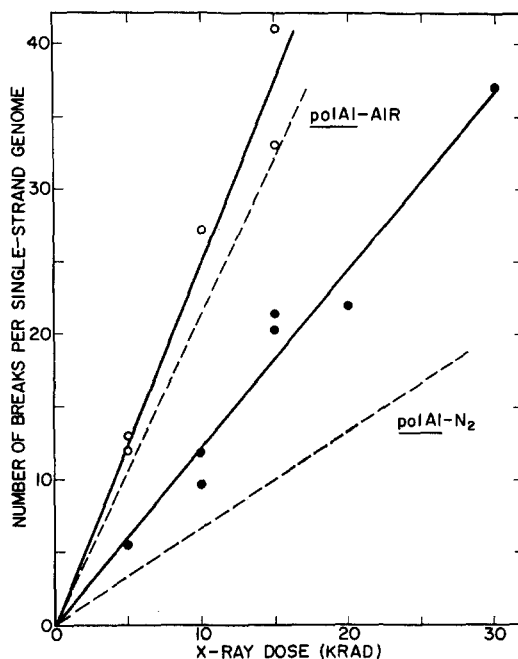


Figure 2. Production of single-strand breaks in *E. coli* K-12 starved of niacin for 18 hours. Cells were irradiated at 0°C in PBS containing 2×10^{-3} M NaCN, and lysed on alkaline sucrose gradients immediately after irradiation. Open symbols, irradiation in equilibrium with air; closed symbols, N_2 . The solid lines are fitted through the origin by linear regression analysis and have slopes of 2.51 and 1.23 breaks per single-strand genome per krad for irradiation in air and N_2 , respectively. The broken lines show the rate of production of breaks in *polA1* cells under comparable irradiation conditions (from Town *et al.* 1972) and have slopes of 2.13 and 0.66 breaks per single-strand genome per krad, respectively.

on the gradients immediately after irradiation (Town *et al.* 1972). The results are shown in figure 2. Reference lines for similar data for *polA1* cells are included for comparison, since they show the numbers of DNA single-strand breaks remaining in untreated cells after ultra-fast repair, but before polymerase repair (Town *et al.* 1972). The results demonstrate that there is a 1.9-fold increase in the yield of DNA single-strand breaks in niacin-starved cells irradiated in the absence of oxygen as compared with *polA1* cells irradiated under the same conditions, while the yield of breaks in aerobically-irradiated niacin-starved cells is only slightly increased (1.2-fold).

No rejoining of single-strand breaks was seen when niacin or NAD was added back to anoxically-irradiated cells.

4. Discussion

Our data show that the pre-irradiation starvation of niacin requiring cells for niacin increases their x-ray sensitivity, in agreement with the observation of Baptist and Friesen (1971).

In addition, niacin starvation caused a 1.9-fold increase in the number of DNA single-strand breaks demonstrable immediately after anoxic irradiation,

but had very little effect on the number of breaks observed in aerobically-irradiated cells. This result is qualitatively similar to our earlier observations (Town *et al.* 1972), which showed that inactivation of cells by heat-treatment or cold shock caused a large increase in the yield of DNA single-strand breaks under anoxic conditions, and a much smaller increase in the yield of breaks under aerobic conditions. Since niacin starvation has been shown to reduce ligase activity in these cells (Nozawa and Mizuno 1969), our results support the idea that DNA ligase is involved in an ultra-fast repair system (Town *et al.* 1972) which rejoins mainly DNA single-strand breaks produced under anoxic conditions.

ACKNOWLEDGMENTS

This work was supported by U.S. Public Health Service research grant CA-06437 and research programme project grant CA-10372 from the National Cancer Institute.

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