The Control of Post irradiation DNA Breakdown in Micrococcus radiodurans

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Received February 24, 1970

INTRODUCTION

Bacteria exposed to x-rays frequently undergo extensive DNA breakdown when incubated post irradiation and this is particularly evident following doses in the lethal range[1-7]. While some correlation can be seen between relative radiosensitivity and extent of DNA breakdown[5,8] it is not clear whether the degradation is either a cause or a consequence of cell death. Treatments which restrict protein synthesis post irradiation have been found to enhance both DNA breakdown[7-10] and the inactivation of bacteria by x-rays[11-13]. The predominant physicochemical alteration in the DNA of x-irradiated cells is the introduction of breaks in the individual nucleotide strands of the twin helix[14-16] and while it is likely that some of these lesions form foci for degradative enzymes the extent to which other structural damage is involved is not known. The exceptionally radioresistant bacterium Micrococcus radiodurans which has a particularly efficient system for the repair of strand breaks in its DNA[17,18] also undergoes DNA breakdown when exposed to 'sublethal' doses

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of ultraviolet light[19] and x-rays[20]. This communication describes experiments which suggest that in M. radiodurans the breakdown of DNA, unless halted, is a lethal process and that termination of degradation is mediated by protein(s) synthesised de novo post irradiation.

Materials and Methods

Exponentially growing cells of M. radiodurans labelled in their DNA with ^{3}H (methyl) thymidine and which had been incubated in non-radioactive TGY (tryptone 0.5%, glucose 0.1%, yeast extract 0.3%) medium for 30 minutes (0.27 generation) to deplete the radioactivity in the acid soluble pool, were exposed at ice bath temperatures to 220 Kvp x-rays in 0.04 M veronal buffer pH 8.6 under anoxia. Following irradiation the cells were collected by centrifugation and then resuspended in prewarmed TGY medium, 0.04 M veronal buffer pH 8.6 or 0.1 M orthophosphate buffer pH 7.0 and incubated at 30°C. Chloramphenicol or Actinomycin D were added to the TGY medium when required to give concentrations of 15 and 5 µg/ml respectively. Aliquots of 0.1 ml were removed during incubation and assayed for tritium activity in (a) the medium or suspending fluid, (b) cold perchloric acid soluble pool and (c) cold acid insoluble DNA by a semi-micro modification of the procedures described by Lett et al[20].

The procedures used for the isolation of DNA from M. radiodurans and for estimation of molecular weight from sedimentation in alkaline sucrose density gradients have been described elsewhere[16]. Estimation of viable counts were made by plating samples on to TGY-agar after suitable dilution in 0.1 M-phosphate buffer pH 7.0.

Results and Discussion

The release of ^{3}H label from the DNA of M. radiodurans following exposure of the cells to 200 krads of x-rays given in anoxia is shown in Figure 1. Under conditions suitable for growth degradation proceeds immediately on incubation, the breakdown products finally appearing in the medium.

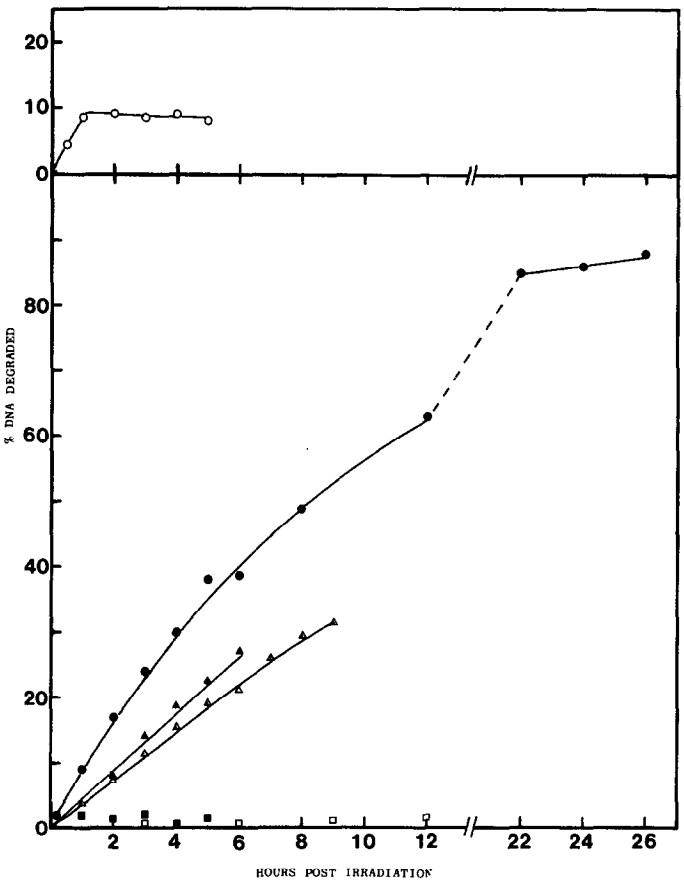

Fig. 1 The post irradiation breakdown of DNA in M. radiodurans following exposure to 200 krads of x-rays in anoxia. The cells were incubated at 30°C in:- O—O, TGY medium; ●—●, TGY medium plus 15μg/ml chloramphenicol; Δ—Δ, TGY medium plus 5μg/ml actinomycin D; ▲—▲, 0.04M veronal buffer pH 8.6; ■—■, 0.1M-phosphate buffer pH 7.0. Unirradiated cells incubated in TGY medium plus 15μg/ml chloramphenicol:- □—□ .

At 60-70 minutes post irradiation the degradation stops and semi-conservative DNA synthesis is observed[17]. When chloramphenicol is added to the growth medium, at a concentration (15 μg/ml) which inhibits protein synthesis, the DNA degradation proceeds initially at the same rate (0.12% per minute). However, degradation does not terminate at 60-70 minutes but proceeds uninterrupted over many hours until more than 80% of the DNA has been rendered acid soluble. Although the level of ^{3}H activity in the acid soluble cell fraction remains constant during the breakdown process, the rate of release of ^{3}H activity into the medium surrounding the cells falls progressively during the later stages of incubation.

The results suggest that the termination of DNA degradation requires protein synthesis post irradiation. This conclusion is supported by the observation that cells exposed to the same dose of x-rays and incubated in either TGY medium containing Actinomycin D (5 μg/ml) or 0.04 M veronal buffer pH 8.6 also show excessive DNA degradation. Under these conditions, however, the rate of DNA degradation is slower than that seen with cells incubated in media containing chloramphenicol. DNA degradation is inhibited however, when irradiated cells are incubated in 0.1 M phosphate buffer pH 7.0 at 30°C and breakdown products do not accumulate in the medium.

From these data we conclude that in M. radiodurans

(1) termination of the DNA breakdown process is mediated by protein(s) synthesised de novo post irradiation.

(2) DNA degradation has no absolute requirement for an exogenous energy supply, since it proceeds in cells incubated in veronal buffer.

(3) The absence of extensive degradation in phosphate buffer

suggests that either phosphate inhibits the degradative enzyme(s) or that a rate limiting step (e.g. the passage of breakdown products across the membrane) may be affected. It is of some interest that the excision of pyrimidine dimers from the DNA of M. radiodurans exposed to ultraviolet light is severely restricted when the cells are incubated in phosphate buffer[19].

M. radiodurans has a particularly efficient system for the repair of x-ray induced lesions in its DNA. Experiments[16,18] have shown that the repair process post irradiation is complex and for the rejoining of broken DNA strands at least three stages can be recognised. Following a dose of 200 krads delivered in anoxia about 50% of the single strand breaks introduced initially into the DNA are restituted during the course of irradiation at $0^{o}C$. Of the breaks remaining at the completion of irradiation, half are rapidly restituted within 5 minutes of incubation post irradiation even by cells incubated in veronal buffer. The broken strands remaining, however, are rejoined more slowly complete restitution taking some 3 hours. The addition of chloramphenicol to the growth medium while not affecting the initial rapid rejoining process prevents the slow rejoining of the remaining broken strands (Figure 2), the molecular weight of the DNA remaining virtually unchanged up to 4 hours post irradiation.

These results indicate that the extensive DNA breakdown observed when protein synthesis is inhibited does not originate from all the radiation induced strand breaks, since about 75% of those formed initially have been repaired by the end of the first 5 minutes post irradiation. While it is clear that some, perhaps all, of the breaks remaining form sites for nucleolytic attack, it is not yet known whether

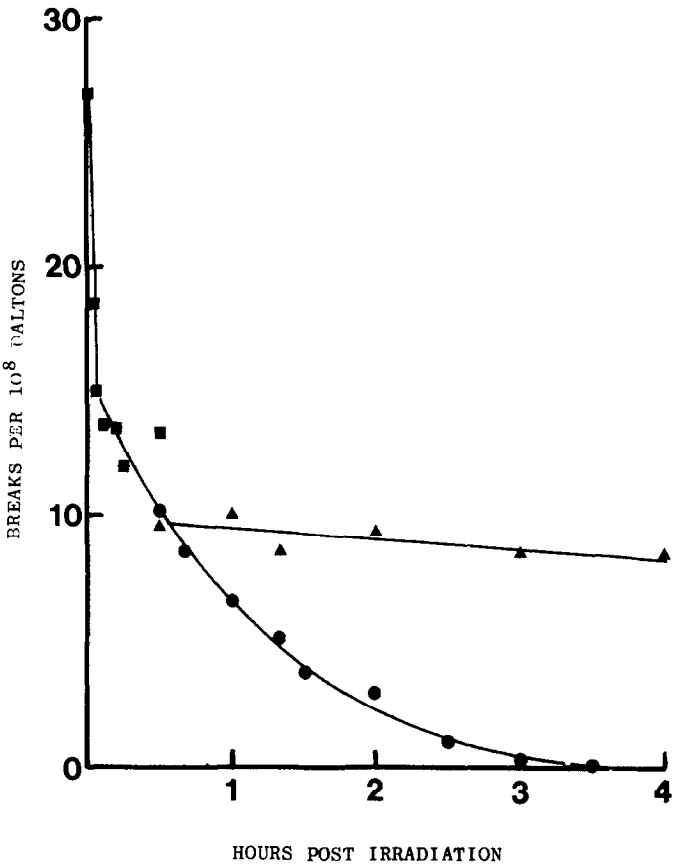

Fig. 2 The influence of suspending medium on the repair of single breaks in the DNA of M.radiodurans exposed to 200 krads of x-rays in anoxia. The cells were incubated in:- ●—●, TGY medium; ▲—▲, TGY medium containing 15μg/ml chloramphenicol; ■—■, 0.04M-veronal buffer pH 8.6.

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such breaks were introduced directly by radiation or if they have arisen, during incubation, as the result of excision of other damage such as defective bases.

Inhibition of protein synthesis post irradiation leads to a progressive loss in viability of M. radiodurans cells exposed to doses of x-rays which are normally not lethal (Figure 3). Under similar conditions, unirradiated cells show no loss in viability during 7 hours incubation in media

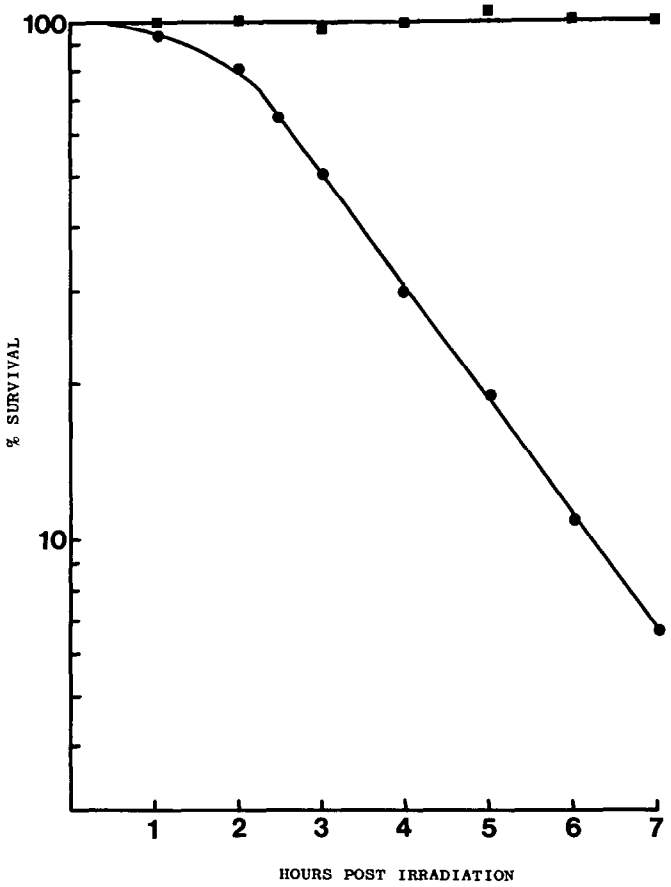

Fig. 3 The influence of chloramphenicol on the survival of M. radiodurans. ■—■ Unirradiated control; ●—●, cells irradiated with 200 krads in anoxia.

containing chloramphenicol. The inactivation of cells irradiated with normally sub-lethal doses of x-rays is also observed when they are incubated in either veronal buffer or TGY medium containing Actinomycin D but the rate of loss in viability is slower. We conclude that the re-establishment of protein synthesis post irradiation is vital not only for the termination of DNA degradation but also for the complete restitution of the DNA damage and ultimately cell survival.

The processes leading to the termination of DNA degradation in M. radiodurans are presently being investigated.

Three possibilities are being considered viz. (1) nucleolytic degradation is halted by the production of a specific inhibitor; (2) the re-establishment of semi-conservative DNA synthesis halts degradation, and (3) termination of DNA degradation follows the re-association of the DNA with the cytoplasmic membrane.

This investigation was supported by a Public Health Service Fellowship from the National Cancer Institute to R.W.S. and by grants to the Chester Beatty Research Institute (Institute of Cancer Research; Royal Cancer Hospital) from the Medical Research Council and the British Empire Cancer Campaign for Research.

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