COLICIN E2-INDUCED DNA SOLUBILISATION IN MUTANTS OF *ESCHERICHIA COLI* DEFICIENT IN ENDONUCLEASE I

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1. Introduction

The protein antibiotic colicin E2 promotes the solubilisation and fragmentation of DNA in *Escherichia coli* without the requirement for the *de novo* synthesis of a DNAase since degradation proceeds even in the presence of chloramphenicol [1, 2]. No nuclease activity of E2 itself has been detected in in vitro experiments [3, 4] and thus the nature of the enzymes involved in DNA degradation in E2-treated cells remains unclear. A suggestion has been made by Almendinger and Hager [5] that colicin E2 causes the redistribution of endonuclease I from the periplasmic space into the interior of the cell where it promotes DNA degradation. These workers provided some circumstantial evidence for this redistribution, and found that a mutant (End^-), deficient in endonuclease I, was less sensitive to E2 than its wild type parent under certain conditions [5]. On the other hand, Obinata and Mizuno [6] using the same mutant strain could find no significant difference in E2-induced DNA solubilisation between the End^+ and End^- strains. There is therefore contradictory evidence on the role of endonuclease I in E2-induced DNA degradation.

We have found that colicin E2-induced solubilisation of DNA occurs in four mutants of *Escherichia coli* deficient in endonuclease I. In two of the mutants the rate of DNA degradation was only slightly reduced compared to that in the wild type parent, whereas in the other two mutants the rate of E2-induced DNA degradation was actually greater than in the wild type strain. It is concluded that endonuclease I is probably not a primary enzyme involved in E2-induced solubilisation of DNA, although the results do not rule out its possible involvement in E2-induced fragmentation of DNA.

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2. Methods

The DNA of the bacteria was uniformly labelled in nutrient broth (NB) containing 100 μCi methyl-[^{3}H] thymine (19 Ci/mmole)/ml in the presence of 5 μgm unlabelled thymine/ml, and 200 μgm 2'deoxyguanosine/ml at 37°C as described previously [2]. The cultures were washed twice by centrifugation in NB + 25 μgm unlabelled thymine/ml and finally suspended in this medium at 5×10^7 cells/ml. After 10 min incubation at 37°C the cells were put on ice. Colicin E2, as a crude preparation [2] was added and the cells were incubated with aeration at 37°C. 0.5 ml samples were taken at intervals into 0.5 ml 10% w/v trichloroacetic acid on ice, and acid-soluble radioactive counts were determined as described by Howard-Flanders and Theriot [7]. As a control, one culture was incubated without colicin.

3. Results and discussion

The amount of E2-induced DNA solubilisation was measured in several different mutants deficient in endonuclease I, isolated and kindly donated by Dr. M. Wright [8]. These strains have mutations either in the

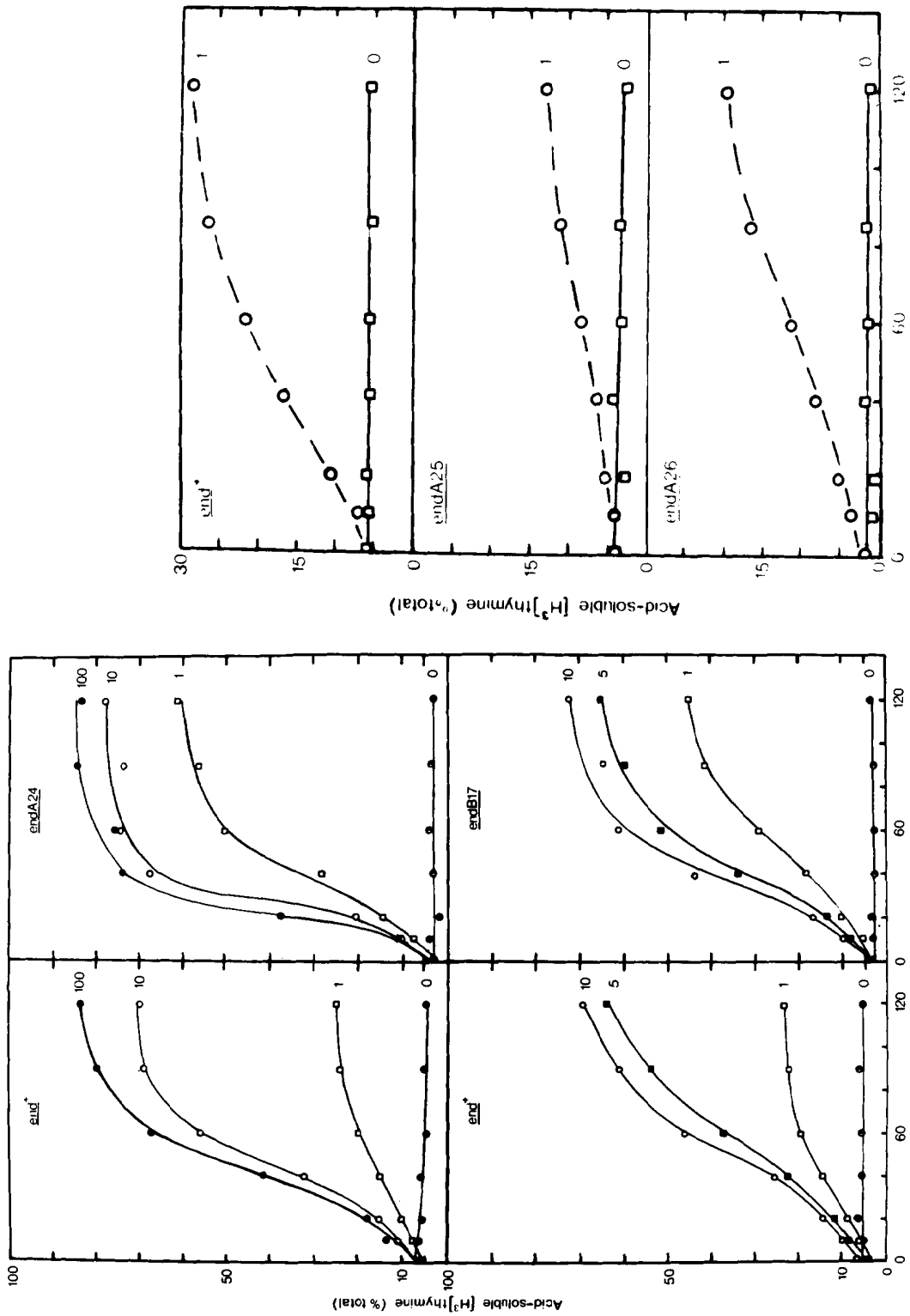

Fig. 1. Colicin E2-induced DNA degradation in End$^+$ and End$^-$ strains of *Escherichia coli* K-12. The experiments were carried out as described in Methods. Acid-soluble radioactivity is shown as a percentage of the total incorporated radioactivity [7]. Figures against the curves refer to the E2 concentration (arbitrary units per 10^7 bacteria). The strains used were HfrH (*end*$^+$) and its End$^-$ derivatives MRW17 (*trp endB17*), MRW24 (*bio endA24*), MRW25 (*endA25*) and MRW26 (*ilv endA26*).

endA gene (at position 57 on the *Escherichia coli* chromosome), or in the *endB* gene (located between positions 13 and 25 [8]). Two of the strains have *amber end* mutations (*endA24* and *endB17*), and three of them (MRW17, MRW25 and MRW26) are reported [8] to have specific activities of endonuclease I at least 100 times less than the wild type End$^+$ strain. The crude colicin E2 preparations used do not appear to contain endonuclease activity, since no double-strand or single-strand breaks can be detected in phage λ DNA treated with E2 in vitro; treatment of the colicin preparation with pancreatic ribonuclease prior to treatment of λ DNA similarly failed to reveal any deoxyribonuclease activity (V. Darby, personal communication).

Almendinger and Hager [5] reported that differences in survival between an End$^-$ and an End$^+$ strain on treatment with colicin E2 could only be observed at low multiplicities and they suggested that as previous workers [6] had used high colicin multiplicities, this would have obscured any differences between the two strains. Therefore in the experiments described below, cultures were treated with a range of concentrations of E2 to give as little as 25%, 70% or greater than 80% solubilisation of DNA in the wild-type strain. The DNA of the bacteria was uniformly labelled with methyl-[^{3}H] thymine (fig. 1) and the appearance of acid-soluble radioactivity was determined after the addition of colicin E2. The data in fig. 1 indicate that rather than showing reduced DNA degradation, two End$^-$ strains (carrying *endA24* and *endB17*) actually showed increased rates of colicin E2-induced DNA degradation compared to the wild type End$^+$ parent. However, in two other End$^-$ strains (carrying *endA25* and *endA26*) degradation was slightly reduced compared to the End$^+$ parent.

The nature of the increased DNA breakdown after E2 treatment in some of the End$^-$ mutants is somewhat mystifying. The most probable explanation is that these strains contain other mutations besides *end*$^-$, which affect the response to colicin E2, since it is known that auxotrophic mutations (e.g., *bio, trp, ilv*) were also induced at the time of mutagenesis by ethyl methane sulphonate [8]. Since colicin E2-induced DNA solubilisation still occurred in cells reportedly having greatly reduced endonuclease I activity [8], a reasonable conclusion is that endonuclease I is not the primary enzyme involved in the direct promotion of DNA solubilisation. It is still conceivable, albeit unlikely, that endonuclease I is normally involved but that in its absence there is an equally alternative pathway of E2-induced DNA solubilisation. The experiments reported above do not exclude the possibility that endonuclease I causes extensive DNA fragmentation in E2-treated cells. However, if this is the case it seems unlikely that such fragments are the major substrate for the exonuclease-like activity which can proceed in E2-treated End$^-$ mutants.

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