Microcin B17 Blocks DNA Replication and Induces the SOS System in *Escherichia coli*

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(Received 14 June 1985; revised 30 August 1985)

Microcin B17 is a novel peptide antibiotic of low M_r (about 4000) produced by *Escherichia coli* strains carrying plasmid pMccB17. The action of this microcin in sensitive cells is essentially irreversible, follows single-hit kinetics, and leads to an abrupt arrest of DNA replication and, consequently, to the induction of the SOS response. RecA^- and RecBC^- strains are hypersensitive to microcin B17. Strains producing a non-cleavable SOS repressor (*lexA1* mutant) are also more sensitive than wild-type, whereas strains carrying a mutation which causes constitutive expression of the SOS response (*spr-55*) are less sensitive to microcin. Microcin B17 does not induce the SOS response in cells which do not have an active replication fork. The results suggest that the mode of action of this microcin is different from all other well-characterized microcins and colicins, and from other antibiotics which inhibit DNA replication.

INTRODUCTION

The name microcins was given (Asensio *et al.*, 1976) to a group of low molecular weight antibiotics produced mainly by *Enterobacteriaceae* of faecal origin. They are considerably smaller than the most extensively characterized colicins, and their production, unlike most of the colicins, is non-lethal for the producing cell, and is not stimulated by agents which induce the SOS response (Baquero & Moreno, 1984). In most cases microcin production is plasmid-dependent and, hitherto, five types of microcins have been identified by cross-immunity, biochemical and genetic criteria (Baquero & Moreno, 1984; Baquero *et al.*, 1981).

Microcin B17 is the prototype of microcins belonging to the immunity group denominated B (Baquero & Moreno, 1984). It is produced by *Escherichia coli* strains harbouring the plasmid pMccB17, previously referred to as pRYC17 (Baquero *et al.*, 1978; Hernández-Chico *et al.*, 1982). This 70 kb plasmid is conjugative and belongs to incompatibility group FII (Baquero *et al.*, 1978). Genetic determinants for microcin B17 functions (production and immunity) are located on a *Bam*HI–*Eco*RI fragment of 5.1 kb which has been cloned into plasmid pBR322. Genetic analyses of mutants defective in microcin production and/or immunity have shown that the synthesis of microcin B17 requires a segment of about 3.5 kb, and that immunity depends on an adjacent segment of about 1 kb (San Millán *et al.*, 1985*a*). At least four cistrons located in the 3.5 kb fragment are involved in microcin B17 production (San Millán *et al.*, 1985*b*). Besides the products of these genes, normal microcin B17 production also requires products encoded by three chromosomal loci (Baquero & Moreno, 1984), one of which is the *ompR* gene product (Hernández-Chico *et al.*, 1982).

In this paper we present studies concerning the mode of action of microcin B17. The results indicate that microcin B17 is a peptide antibiotic which inhibits DNA elongation and induces expression of the SOS network.

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Table 1. *E. coli* K12 strains used

Strain	Genetic characters	Reference or source*
BM21	GyrA ⁻ (λ^+)	Hernández-Chico <i>et al.</i> (1982)
MC4100	<i>araD139</i> <i>NlacU169</i> RpsL ⁻ RelA ⁻ ThiA ⁻	Casadaban (1976)
GS7	As MC4100, <i>malE::Tn5</i>	Guarente <i>et al.</i> (1980)
SM103	As MC4100, <i>proC::Tn5</i>	J. Beckwith
RYC511	As MC4100, pColE2-P9	} Hernández-Chico <i>et al.</i> (1982)
pop3351	As MC4100, Δ <i>malB1</i>	
RYC361	As pop3351, ThyA ⁻	Spontaneous ThyA ⁻ mutant of pop3351 (trimethoprim selection)
RYC893	As pop3351, pMM102	} San Millán <i>et al.</i> (1985a)
RYC895	As pop3351, pMM120	
RYC726	GyrA ⁻ Δ (<i>sbmA-pho</i>)14 RpoB ⁻ (λ^+)	Spontaneous microcin B17-resistant mutant
AB1157	Thr ⁻ Leu ⁻ Pro ⁻ <i>his-4</i> <i>argE3</i> Thi ⁻ Lac ⁻ Gal ⁻ RpsL ⁻ <i>sup-37</i>	Howard-Flanders & Theriot (1966)
GY753	As AB1157, UvrA ⁻	} M. Blanco
GY773	As AB1157, Arg ⁺ <i>metB1</i> <i>lexA1</i>	
GY795	As AB1157, <i>recB21</i>	
GY759	As AB1157, Thr ⁺ Leu ⁺ Arg ⁺ ThyA ⁻ <i>uvrA6</i> <i>recA13</i>	
GY2682	HfrH KL16 Thr ⁻ Ile ⁻ Val ⁻ Thi ⁻ <i>recB21</i> <i>recC22</i>	
HfrH	GalE ⁻	J. Beckwith
RYC380	As HfrH, <i>recA56</i> <i>srl-300::Tn10</i>	Tc ^R RecA ⁻ transductant of HfrH; donor RYC816
GC2386	As AB1157, <i>recF143</i> [λ d(<i>recA::lac</i>)clind]	} Casaregola <i>et al.</i> (1982a)
GC2421	As AB1157, <i>recB21</i> [λ d(<i>recA::lac</i>)clind]	
GC2241	HfrH <i>lacU169</i> RelA ⁻ Thi ⁻ (Mu) <i>recA::Mud</i> (Ap ^R <i>lac</i>) (λ precAcIind)	
GC2321	As GC2241, <i>pyrD::Tn5</i> <i>lexA55</i> <i>sfiA11</i>	
GC2181	HfrH <i>NlacU169</i> RelA ⁻ Thi ⁻ (Mu) <i>sfiA::Mud</i> (Ap ^R <i>lac</i>)	
GC4572	As GC2181, <i>lexA55</i> <i>malB::Tn9</i>	} Huisman & D'Ari (1981)
GC4415	Thr ⁻ Leu ⁻ His ⁻ PyrD ⁻ <i>trp::MuC</i> ⁺ Δ <i>lac</i> MalB ⁻ GalK ⁻ Str ^R <i>srl-300::Tn10</i> <i>sfiA::Mud1</i> (Ap ^R <i>lac</i>)	
GC4425	As GC4415, Srl ⁺ <i>recA1</i> Tc ^S	
GC4421	As GC4415, <i>lexA1</i>	
RYC351	As GC4415, ThyA ⁻	
RYC352	As GC4415, <i>recB21</i> <i>recC22</i>	Spontaneous ThyA ⁻ mutant GC4415 (trimethoprim selection)
RYC353	As GC4415, <i>proC::Tn5</i>	Thy ⁺ RecBC ⁻ transductant of RYC351; donor GY2682
RYC354	As GC4415, Δ (<i>sbmA-phoA</i>)14	Km ^R Pro ⁻ transductant of GC4415; donor SM103
GW1103	Thr ⁻ Leu ⁻ <i>his-4</i> ArgE ⁻ Δ <i>lacU169</i> <i>recA441</i> UvrA ⁻ <i>sfiA11</i> <i>umuC::Mud</i> (Ap ^R <i>lac</i>)	Pro ⁺ Pho ⁻ microcin-resistant transductant of GC4415; donor RYC726
RYC382	As GW1103, UvrA ⁺ <i>malE::Tn5</i>	Bagg <i>et al.</i> (1981)
GC2405	Thr ⁻ Leu ⁻ Pro ⁻ His ⁻ Ilv ⁻ Thi ⁻ Lac ⁻ Gal ⁻ RpsL ⁻ <i>sup-37</i> <i>srl-300::Tn10</i> [λ d(<i>recA::lac</i>)clind] <i>dnaA46</i>	} Casaregola <i>et al.</i> (1982b)
GC2391	As GC2405, Thr ⁺ DnaA ⁺ <i>dnaC325</i>	

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METHODS

Bacterial strains. The strains of *E. coli* used are listed in Table 1. Plasmid pColE2-P9 determines synthesis of colicin E2 and immunity to this colicin (Mock & Pugsley, 1982). Plasmid pMM102, which determines overproduction of microcin B17, is a pBR322 derivative in which the 375 bp *EcoRI*-*Bam*HI fragment (Bolívar *et al.*, 1977) has been replaced by a 5.1 kb *EcoRI*-*Bam*HI fragment, from pMccB17, encoding microcin B17 and immunity to it (San Millán *et al.*, 1985a). pMM120 is a pMM102 derivative lacking a 0.8 kb *Hind*III fragment required for production of microcin.

Media. M63 minimal salts medium (Miller, 1972) supplemented with 1 μ g thiamin ml⁻¹ and 0.2% (w/v) glucose or 0.4% (v/v) glycerol and, when appropriate, with L-amino acids (20 μ g ml⁻¹) and thymine (20 μ g ml⁻¹), or LB medium (Miller, 1972) were used throughout. Solid media contained 1.5% (w/v) Difco agar. Bacterial strains were

propagated on LB plates. Unless otherwise noted, experiments with microcin were done in M63 medium. Antibiotics were used at the following concentrations ($\mu\text{g ml}^{-1}$): 30, ampicillin (Ap); 30, tetracycline (Tc); 100, streptomycin (Sm); 50, nalidixic acid (Nal). Phage P1 was grown on LB plates supplemented with 25 mM- CaCl_2 , 10 mM- MgSO_4 , 0.2% glucose and 1% Difco agar. Lambda phage was assayed on LB plates lacking yeast extract and containing 1.2% agar. 5-bromo-4-chloro-3-indolyl β -D-Galactoside (X-gal) was used at $40 \mu\text{g ml}^{-1}$.

Genetic methods. Transformation with plasmid DNA was done as described by Dagert & Ehrlich (1979). Trimethoprim selection of ThyA^- mutants and P1vir-mediated transduction were carried out as described by Miller (1972). The marker *recA56* was routinely introduced into strains by cotransduction with the marker *srI::Tn10*. Tetracycline resistant bacteria were selected. Clones which were also *recA56* were identified by UV hypersensitivity. Markers *recB* and *recC* were introduced into ThyA^- bacteria by selecting for Thy^+ ; these clones were then tested for microcin B17 sensitivity/resistance (RecB^- and RecBC^- mutants are very sensitive to microcin B17, see later).

Preparation of microcin B17 extracts. Microcin was obtained from solid medium using the producing strain RYC893. Bacteria grown on LB agar containing Ap were resuspended in 5 ml M63 medium to a density of about 10^8 cells ml^{-1} . Samples (0.1 ml) were mixed with 3 ml soft agar and poured into plates containing 25 ml M63 agar supplemented with 0.4% glycerol, the surface of which had been covered with a cellophane membrane. The plates were incubated for 40 h at 37°C . Then the bacteria in the overlay were removed with the membrane and the remaining agar was frozen at -20°C for 24 h to destroy the gel. After thawing at room temperature, the contents of the plates were recovered and centrifuged at 4°C in a Beckman JA-20 rotor (10000 r.p.m., 30 min). The supernatant was passed through a Millipore filter (HAWP45) and the filtrate was separated into 3 ml samples and maintained at -20°C until use. Extracts devoid of microcin (for use as negative controls) were obtained by the same method, using strain RYC895 Mcc^- .

Determination of microcin B17 activity. The antibiotic activity in cell extracts was determined by the critical dilution method (Mayr-Harting *et al.*, 1972). Extracts were diluted in M63 minimal salts medium and $10 \mu\text{l}$ samples were spotted on M63 glucose plates overlaid with 3 ml soft agar containing approximately 2×10^7 indicator bacteria (strain BM21). After overnight incubation at 37°C , plates were examined for growth inhibition. The antibiotic activity was expressed as units ml^{-1} (AU ml^{-1}), one unit being the quantity of microcin present in the $10 \mu\text{l}$ sample of the highest dilution able to produce a clear halo of inhibition. Our preparations routinely contained approximately 1000 AU ml^{-1} .

Microcin B17 survival studies. Survival following microcin B17 treatment was determined by diluting cells and immediately plating them onto LB agar.

Measurement of protein, RNA and DNA synthesis. To examine the effect of the microcin on protein synthesis, an exponentially growing AB1157 culture (OD_{600} 0.4) was divided between two flasks. Microcin B17 (200 AU ml^{-1}) was added to one of them, and both cultures were incubated for 7 min before adding [^3H]leucine ($2 \mu\text{Ci ml}^{-1}$; specific activity 102 Ci mmol^{-1}) (1 Ci = 37 GBq). At various times, 0.5 ml samples were pipetted into 1.5 ml 7.5% (w/v) cold trichloroacetic acid (TCA) and held at 4°C for 1 h. The mixtures were then heated at 90°C for 30 min and filtered through Millipore (HAWP45) membranes. When dry, the filters were counted in a scintillation counter. Determination of RNA synthesis in microcin-treated AB1157 cells was done in a similar way. The cells were labelled with [^3H]uridine ($2 \mu\text{Ci ml}^{-1}$; specific activity 30 Ci mmol^{-1}) and the heating step before the filtration step was omitted.

The synthesis of DNA by AB1157 and RYC361 cells growing in the presence of microcin B17 was determined in M63 glucose medium containing [*methyl*- ^3H]thymidine ($3 \mu\text{Ci ml}^{-1}$; specific activity 45 Ci mmol^{-1}) and deoxyadenosine ($200 \mu\text{g ml}^{-1}$). For ThyA^- cells, unlabelled thymine was added to $20 \mu\text{g ml}^{-1}$ instead of deoxyadenosine. Samples taken at various intervals were treated as for the determination of RNA synthesis.

The effect of microcin B17 and nalidixic acid on the rate of DNA synthesis was measured in cells growing in M63 glucose medium. Cultures (OD_{600} 0.2) were divided into two or more fractions which were treated with various quantities of either microcin or nalidixic acid. Controls were either not treated or were treated with a non-microcin extract. At various intervals 0.3 ml samples of the cultures were pulse-labelled for 3 min with [*methyl*- ^3H]thymidine ($3.3 \mu\text{Ci ml}^{-1}$; specific activity 45 Ci mmol^{-1}). Labelling was stopped by the addition of 4 ml ice-cold 5% (w/v) TCA. TCA-precipitable counts were measured as above. The rates were expressed as a percentage of the untreated control.

Microcin B17-induced DNA degradation. Cells of strain RYC361 were grown in M63 glucose, supplemented with thymine ($20 \mu\text{g ml}^{-1}$), at 37°C . When the culture reached OD_{600} 0.3, [*methyl*- ^3H]thymidine ($3 \mu\text{Ci ml}^{-1}$; specific activity 45 Ci mmol^{-1}) was added and incubation was continued for 90 min. The cells were washed twice in M63 buffer and resuspended in the same volume of M63 glucose medium supplemented as above. The suspension was divided into two parts and microcin (200 AU ml^{-1}) was added to one of them. To measure the stability of DNA we essentially followed the method of Howard-Flanders & Theriot (1966). Samples (0.2 ml) were taken at intervals and mixed with an equal volume of cold 10% (w/v) TCA. Albumin (0.01%) was added to increase the bulk of the precipitate. After 1 h at 0°C , the mixtures were centrifuged at 12000 r.p.m. for 10 min. Supernatants contained the cold-soluble material. The pellets were dispersed in 0.4 ml of 5% (w/v) TCA and heated to 90°C for 30 min. The

mixtures were centrifuged to eliminate the remaining insoluble material and the supernatant, containing the cold acid-insoluble material, was kept. Samples (50 μ l) of cold acid-soluble and acid-insoluble fractions were placed onto GF/C Whatman filters and the radioactivity was counted.

β -Galactosidase assay. β -Galactosidase was assayed and activity expressed according to Miller (1972).

Prophage and colicin induction. Induction of lambda was studied as follows: cultures of BM21 (λ^+) were grown at 37 °C in M63 medium to a density of 10^8 cells ml⁻¹ and then centrifuged; the cell pellet was resuspended in the same volume of fresh M63 medium. Cultures were divided into two parts, one of which was treated with microcin (50 AU ml⁻¹), and the other with an equivalent volume of an extract that lacked microcin. After incubation for 10 min, the cultures were diluted 1:1000 in LB and incubated for 2 h at 37 °C. At various times, samples of the cultures were plated into LB agar medium to determine the number of c.f.u. and the number of p.f.u., using MC4100 as the lambda indicator strain. Colonies and plaques were counted 20 h later.

Microcin B17-induced mutability. The effect of microcin B17 on the rate of spontaneous mutagenesis was determined by looking for mutants in the lactose and galactose operons in a GalE⁻ background (Miller, 1972). Bacteria were grown to OD₆₀₀ 0.6 in M63 medium. Microcin (100 AU ml⁻¹) was added to the culture and survival at various intervals was determined as above. At the same time, 0.1 ml samples were diluted to 10 ml M63 medium (the concentration of microcin was thereby reduced 100-fold) and the diluted cultures were incubated at 37 °C until the stationary phase was reached. Cells were then plated on M63 agar + 0.2% glucose to measure viability, and on either M63 agar + 0.2% glycerol + 0.02% lactose or M63 agar + 0.2% glucose + 0.03% phenyl β -D-galactoside + 10 mM-IPTG to count Lac⁻ and/or Gal⁻ mutants. Plates were incubated at 42 °C to avoid growth of mucoid colonies. The frequency at which mutants were detected after various periods of treatment with microcin B17 was expressed as the ratio of the numbers of mutants and total viable counts.

RESULTS

Effects of microcin B17 on sensitive cells

We treated exponentially growing *E. coli* AB1157 in M63 glucose medium with 200 AU microcin ml⁻¹ (which is sufficient to reduce viability to less than 0.1% in 10 min) and measured cell growth and macromolecular syntheses. Bacterial mass (OD₆₀₀) increased normally for 45 min, after which growth slowed and finally stopped after 100–120 min. From this time, slight decreases in optical density of the culture were sometimes observed (data not shown). Protein and RNA synthesis (as measured by incorporation of [³H]leucine and [³H]uridine respectively) continued normally for at least 45 min, but DNA synthesis (incorporation of [*methyl*-³H]thymidine) was immediately reduced, and stopped after 25–30 min (data not shown).

These results suggested that microcin B17 specifically inhibited chromosome replication. To confirm this, we examined the rate of DNA synthesis after various periods of incubation in the presence of microcin B17 by using pulse-labelling experiments. Microcin provoked a rapid decline in DNA synthesis, with the rate of inhibition being dependent on the microcin concentration (Fig. 1). In the presence of 200 AU microcin ml⁻¹, DNA synthesis was reduced by 90% after 20 min treatment. Similar kinetics of inhibition were obtained with cultures treated with 10 μ g sodium nalidixate ml⁻¹ (Fig. 1), which inhibits DNA replication by inactivating subunit A of DNA gyrase (Sugino *et al.*, 1977). Microcin-induced inhibition of DNA synthesis was followed after 30 min treatment by the continuous release of incorporated [*methyl*-³H]thymidine into acid soluble fragments, which indicated that DNA degradation had occurred. Killing by microcin B17 followed single-hit kinetics, indicating that a single molecule of microcin can, with a certain probability, kill a cell without the cooperation of other microcin molecules. When microcin-treated cultures were examined by light microscopy, long filaments were observed.

Microcin B17 induces the SOS response

Treatment with agents which damage DNA or which cause the premature termination of DNA replication elicits the so-called SOS response in *E. coli* (Gudas & Pardee, 1975; Little & Mount, 1982; Radman, 1974; Walker, 1984; Witkin, 1976).

In order to test whether treatment with microcin B17 elicited the SOS response, we examined its effects on the expression of hybrid operons in which the β -galactosidase gene, *lacZ*, was controlled by an SOS-regulated promoter (*sfiAp*, *recAp* or *umuCp*). We also examined the effect of microcin treatment on prophage and colicin E2 induction. We found, as expected, that

Fig. 1

Fig. 2

Fig. 1. Effect of different concentrations of microcin B17 and nalidixic acid on the rate of synthesis of DNA. Cultures of pop3351 were grown exponentially in M63 glucose medium. At time zero, antibiotics were added to subcultures and incubation continued for 45 min. At various times samples were removed and pulse-labelled with [*methyl*-³H]thymidine for 3 min. For further details see Methods. ●, Microcin B17 (25 AU ml⁻¹); ○, microcin B17 (200 AU ml⁻¹); ▲, sodium nalidixate (10 µg ml⁻¹); △, sodium nalidixate (40 µg ml⁻¹).

Fig. 2. Lack of induction of *sfiA* expression by microcin B17 in RecBC⁻ cells. Cultures of isogenic strains GC4415 (*sfiA-lacZ* RecBC⁺) and RYC352 (*sfiA-lacZ* RecBC⁻) growing exponentially in M63 medium supplemented as required were divided into three portions. Microcin B17 (100 AU ml⁻¹) was added to the first subculture, nalidixic acid (40 µg ml⁻¹) to the second and bleomycin (40 µg ml⁻¹) to the third. Incubation was continued and β-galactosidase activity was determined at the indicated times. Solid lines: RecBC⁺ + microcin B17 (○); RecBC⁺ + nalidixic acid (△); RecBC⁺ + bleomycin (□). Broken lines: RecBC⁻ + microcin B17 (●); RecBC⁻ + nalidixic acid (▲); RecBC⁻ + bleomycin (■).

Table 2. Induction of the expression of *recA*, *sfiA* and *umuC* in the presence of microcin B17

Cells were grown at 37 °C to exponential phase (OD₆₀₀ 0.2) in M63 medium supplemented as required. At time zero, microcin B17 (100 AU ml⁻¹) was added to one portion of each culture, and an equivalent volume of non-microcin extract to the rest of each culture. β-Galactosidase activity [units according to Miller (1972)] was assayed at 0, 60 and 120 min. The values are means of three independent experiments, the results of which agreed to within 8%.

<i>E. coli</i> strain	Operon fusion	Microcin B17	β-Galactosidase activity (Miller units)		
			0 min	60 min	120 min
GC2241	<i>recA-lacZ</i>	—	450	490	530
		+	450	3700	5300
GC4415	<i>sfiA-lacZ</i>	—	30	27	36
		+	30	550	980
RYC382	<i>umuC-lacZ</i>	—	7	9	8
		+	7	36	50

treatment with microcin B17 increased all of these activities (Table 2). Treatment with nalidixate (20 µg ml⁻¹) and bleomycin (30 µg ml⁻¹) gave similar results to those of Table 2 (data not shown). A 2000-fold increase from the basal colicin E2 level was found when MC4100(pColE2-P9) cells were cultivated for 100 min in the presence of 50 AU microcin ml⁻¹. When prophage induction was examined in strain BM21 (λ⁺), as stated in Methods, we found that 10 min incubation was sufficient to convert 50% of the c.f.u. into infective centres, the mean burst size being 200. In contrast, microcin B17 did not induce the SOS response in strains RYC726 and RYC354, which were resistant to the killing effect of the microcin B17, and control extracts from cultures of a non-producing strain showed no effect.

Fig. 3

Fig. 4

Fig. 3. Kinetics of *sfiA* induction following short and continuous treatments with microcin B17 and nalidixic acid. A culture of strain GC4415 (*sfiA-lacZ*), grown exponentially at 37 °C in supplemented M63 medium, was washed, resuspended in fresh medium and divided in two portions. At time zero, microcin B17 (200 AU ml⁻¹) was added to one portion and nalidixic acid (20 µg ml⁻¹) to the other. After 5 min, half of each culture was washed and resuspended in antibiotic-free fresh medium. The other half of each culture was maintained in the presence of the respective antibiotic. β -Galactosidase activity was determined in the four subcultures at the indicated times. Open symbols, continuous treatment. Filled symbols, 5 min treatment. Centrifugation removed all the antibiotics, since sensitive MC4100 (Muc⁺) cells were not killed at all, but grew normally when they were incubated for 15 min in resuspended cultures.

Fig. 4. Survival of different SOS mutants after microcin B17 treatment. Bacterial cultures grown exponentially in M63 (2 × 10⁸ cells ml⁻¹) were treated with microcin B17. At various times, diluted samples were spread on LB plates and incubated overnight at 37 °C to determine viability. Isogenic strains AB1157 wild-type (○), GY773 *lexA1* (●), GY759 RecA⁻ (△) and GY795 RecB⁻ (▲) (solid lines) were treated with 30 AU microcin B17 ml⁻¹. Isogenic strains GC2181 SfiA⁻ (□) and GC4572 SfiA⁻ *spr-55* (■) (broken lines) were treated with 100 AU microcin B17 ml⁻¹.

The normal SOS response cannot be triggered if the cells do not have a functional *recA* gene or if the *lexA* gene encodes a non-cleavable repressor (as is the case with the *lexA1* mutation). Microcin B17 treatment did not induce the SOS system in strains bearing these mutations (GC4425, GC4421), indicating that the antibiotic induces the SOS response by the normal pathway.

Microcin B17 and nalidixic acid both seem to induce the SOS response by blocking DNA replication. SOS induction by nalidixic acid and coumermycin, which specifically inhibits the B subunit of DNA gyrase, requires the action of exonuclease V, encoded by the *recB* and *recC* genes (Casaregola *et al.*, 1982a; Gudas & Pardee, 1976; Smith, 1983); this was also found to be the case for microcin B17 (Fig. 2). The SOS response was not induced following treatment of GC2421 RecB⁻ cells with microcin B17 (data not shown). Other inducers of the SOS response such as mitomycin C, which crosslinks DNA strands, bleomycin, which causes single- and double-strand breaks in DNA, and ultraviolet light do not require the RecBC function, but the *recF* gene product is apparently needed for maximum induction of the SOS response in the UV-irradiated cells (Clark *et al.*, 1978; McPartland *et al.*, 1980). A mutation in *recF* (strain GC2386) did not affect expression of the *recA-lacZ* fusion by microcin B17, indicating that SOS induction by microcin B17 is *recF*-independent (data not shown).

An important difference between the effect of microcin B17 and nalidixic acid was revealed by studying the effect of short periods of exposure of these inducers on ϕ (*sfiA-lacZ*) expression (Fig. 3). Our results show that 5 min exposure to the microcin is sufficient to obtain full

Table 3. Inducibility of *recA* expression in a *DnaA*⁻ (Ts) initiation mutant

Cells of GC2405 (*dnaA46 recA-lacZ*) were grown in LB medium at 30 °C until they reached 5×10^7 cells ml⁻¹. Portions of the culture were maintained at 30 °C with or without antibiotics for 120 min, and then assayed for β -galactosidase (expt 1). Other portions were transferred to 42 °C with or without antibiotic for 90 min, and then assayed for β -galactosidase (expt 2). At this time the 42 °C culture without antibiotic was divided into four parts, three of which received an antibiotic. Incubation was continued at 42 °C for 60 min, and then β -galactosidase assayed (expt 3). Units of β -galactosidase activity are expressed according to Miller (1972). The values are means of two independent experiments, the results of which agreed to within 10%. *t*₀, Time of addition of antibiotic.

Expt	β -Galactosidase activity (Miller units)			
	None	Bleomycin (40 μ g ml ⁻¹)	Antibiotic: Nalidixic acid (40 μ g ml ⁻¹)	Microcin B17 (200 AU ml ⁻¹)
1. <i>t</i> ₀ + 120 min, 30 °C	830	3210	3110	3340
2. <i>t</i> ₀ + 90 min, 42 °C	690	1750	1100	1070
3. 90 min, 42 °C; <i>t</i> ₀ + 60 min, 42 °C	690	1790	540	680

induction. We concluded that the effect of microcin B17 was essentially irreversible, whereas the effect of nalidixic acid was negligible if the antibiotic was removed after only 5 min. Notice that at this time the rate of DNA synthesis had been reduced by 50% with both antibiotics (see Fig. 1).

Microcin B17 requires an active replication fork to induce SOS gene expression

The effects of bleomycin, nalidixic acid and microcin B17 on ϕ (*recA-lacZ*) expression were compared in strains carrying a *DnaA*⁻ (Ts) mutation, in which new rounds of chromosome replication are not initiated when cells are shifted from 30 °C to 42 °C, although cycles of replication begun prior to the temperature shift are completed. Incubation at 42 °C does not induce the SOS response in the cells because DNA replication is not prematurely arrested (Casaregola *et al.*, 1982*b*; Gudas & Pardee, 1976; Monk & Gross, 1971; Schuster *et al.*, 1973). The ability of microcin B17 and nalidixic acid to induce ϕ (*recA-lacZ*) expression was markedly reduced shortly after the shift to 42 °C and was completely abolished after prolonged incubation, when all of the cells would have completed their pre-initiated cycles of replication (Table 3). Induction of the *recA* gene was still possible, however, with bleomycin, even after prolonged incubation at 42 °C as previously reported (Satta & Pardee, 1978). It should be noted that mitomycin C (Casaregola *et al.*, 1982*b*) and UV irradiation (Little & Hanawalt, 1977) also induce *RecA* synthesis in these conditions. The three antibiotics, microcin B17, nalidixic acid and bleomycin, were unable to induce *recA* expression in strain GC2391 *DnaC*⁻ (Ts) at 42 °C (data not shown). This result suggests that induction mediated by bleomycin in the *DnaA*⁻ (Ts) mutant is dependent on the *dnaC* gene product. The same conclusion was previously obtained for induction by mitomycin C (Casaregola *et al.*, 1982*b*).

Effects of mutations in SOS-regulated genes on microcin B17 sensitivity

Preliminary experiments employing the critical dilution assay on plates indicated that mutations in *sfiA*, *umuC*, *uvrA*, *uvrB* and *recF* did not affect sensitivity to microcin B17, whereas strains carrying mutations in *recB*, *recBC*, *recA* or *lexA1* were considerably more sensitive than wild-type strains, and a strain carrying the *spr55* (*lexA* defective) mutation, in which SOS gene expression is fully derepressed (Casaregola *et al.*, 1982*a*), was more resistant than wild-type strains. The effects of these latter mutations were confirmed by measuring the microcin B17 sensitivity of isogenic strains in liquid cultures (Fig. 4).

Microcin B17 is mutagenic

The *umuC* and *umuD* genes constitute a LexA-repressed operon (Bagg *et al.*, 1981; Shinagawa *et al.*, 1983; Walker, 1984) which is derepressed following treatment with microcin B17 (see above). Both genes are required for mutagenesis induced by UV light and other agents. It is

interesting, therefore, that we observed a dose-dependent increase in Lac⁻ and Gal⁻ mutations in microcin-treated HfrH GalE⁻ cells, the maximum increase being 10 times in cultures where survival rates were 10⁻⁴ to 10⁻⁵ (data not shown). We do not know whether these induced mutations were caused by mis-repair of lesions caused by microcin or were due to increased *umuCD* expression. In any case, the mutagenic effect was SOS-dependent since no increase of spontaneous mutation frequency was observed in microcin-treated RYC380 RecA⁻ cells (data not shown).

DISCUSSION

In this paper we describe studies on the mode of action of microcin B17. The effects of this microcin on the expression of *recA*, *sfiA* and *umuC* genes, and on colicin E2 production and prophage lambda induction indicate that the SOS system is induced in microcin B17-treated cells. Three sets of results indicate that SOS induction is the result of the primary action of the microcin as an inhibitor of DNA replication: (i) the rate of DNA replication is reduced very soon after the addition of microcin to the cells, whereas extensive DNA degradation was not observed until 30 min after the antibiotic was added; (ii) induction of the SOS response was RecBC dependent, as is the case for other agents which block chromosome elongation, but not for agents which directly affect DNA structure; and (iii) microcin B17 does not induce the SOS response in cells which do not have an active chromosome replication fork. The effects of microcin B17 are, therefore, superficially similar to those produced by treatment with sodium nalidixate, by thymine starvation of strains carrying a ThyA⁻ mutation, and by transferring to the restrictive temperature mutants carrying DnaB⁻ (Ts) mutations (Casaregola *et al.*, 1982*a*; Casaregola *et al.*, 1982*b*; Gudas & Pardee, 1976; Witkin, 1976). We do not yet know whether microcin B17 interacts directly with DNA or with one of the proteins involved in DNA replication, but we do know that the reaction is essentially irreversible in sensitive cells. One approach to study the target of microcin B17 is to characterize mutants with altered sensitivity. We have so far identified three classes of mutants with reduced sensitivity to microcin B17: those which lack the outer membrane protein OmpF; those which are mutated in the *phoA*-linked gene *sbmA* (sensitivity to B17 microcin) (M. Laviña, A. Pugsley & F. Moreno, unpublished); and those carrying mutations in *lexA* (*spr*) as noted above. Strains carrying mutations in *recA*, *recB*, *recBC* or *lexA* (Ind⁻) exhibit increased microcin B17 sensitivity, and are incapable of inducing the SOS response. These results suggest that although one microcin molecule is sufficient to kill a cell, according to single-hit-killing kinetics, at least some potentially lethal events are neutralized by products of one or more of the SOS-regulated genes. This escape mechanism may involve increased production of an enzyme whose action could be to remove microcin B17 from its site of action, or to repair a microcin-induced lesion.

The numerous differences between microcins and the more extensively characterized colicins have been mentioned here and elsewhere (Baquero & Moreno, 1984). Further characterization of the microcin B17 molecule is severely hampered by the very low yields obtained, even from over-producing strains. Experiments with crude extracts and with partially purified preparations obtained by hydrophobic column chromatography and gel filtration indicate that microcin B17 has an *M_r* of 4000–5000 (as determined by gel filtration, dialysis and Amicon filtration) and is sensitive to thermolysin, pronase and proteinase K (M. Herrero, M. Laviña, A. Pugsley & F. Moreno, unpublished). These results indicate that microcin B17 is indeed different from most colicins. Other results indicate that microcin B17 may be identical to colicins X-185II and X-CA23 (Davies & Reeves, 1975). Indeed, we have found that colicin X production is not induced by mitomycin C, that colicin X induces the SOS response in treated cells, that microcin B17 and colicin X-producing *E. coli* K12 are cross-immune, that *sbmA* mutants are also resistant to colicin X and that the ColX plasmids have a region of significant homology (as determined by Southern hybridization) with the microcin B17 operon of pMccB17 (J. L. San Millán and coworkers, unpublished). Our present approach to the study of microcin B17 action is to characterize other microcin-insensitive mutants so as to analyse the way in which producing cells protect themselves against the action of their own microcin.

We thank F. Baquero for his enthusiastic support during this work; A. Pugsley for critical comments and very kind help in the English manuscript preparation; C. Hernández-Chico, M. Laviña and J. L. San Millán, of our laboratory, for many useful discussions; R. D'Ari, J. Beckwith, M. Blanco, O. Huisman, A. Pugsley, A. Toussaint and G. Walker for the gifts of strains; S. Jimenez and J. Talavera for technical assistance. This work was financially supported by the FIS (Ministerio de Sanidad). M.H. was supported during this study by a FIS predoctoral fellowship.

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