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The *recA*$^+$ Gene Product Is More Important than Catalase and Superoxide Dismutase in Protecting *Escherichia coli* Against Hydrogen Peroxide Toxicity

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Various deoxyribonucleic acid repair-deficient strains of *Escherichia coli* K-12 were exposed to hydrogen peroxide under anaerobic conditions, and the killing of the strains was determined. The level of catalase, peroxidase, and superoxide dismutase in cell-free extracts of the strains as well as the capacity of intact cells to decompose hydrogen peroxide were assayed. *recA* strains were more rapidly killed than other strains with deoxyribonucleic acid repair deficiencies. There was no correlation between the killing rate of the strains and the capacity of intact cells to decompose hydrogen peroxide or the level of catalase and superoxide dismutase in cell-free extracts. The level of peroxidase in cell-free extract was too low to be determined.

In *Escherichia coli* and *Salmonella typhimurium*, DNA is injured by hydrogen peroxide and some DNA repair-deficient strains are more sensitive to hydrogen peroxide than DNA repair-proficient strains (1, 10, 18, 19, 20). It has not been established whether these differences in sensitivity to hydrogen peroxide result from the various levels in the cell of the protecting enzymes catalase, peroxidase, or superoxide dismutase.

We tested the sensitivity to hydrogen peroxide of various DNA repair-deficient strains of *E. coli* K-12 and found that the sensitivity of the strains to hydrogen peroxide was not correlated with their cellular levels of catalase or superoxide dismutase. The experiments were run under anaerobic conditions to keep the protecting enzymes on constitutive levels and to prevent the formation of hydrogen peroxide in the media by autoxidation (5, 6, 9).

Strains of *E. coli* K-12 used are listed in Table 1. All strains were stored on blood agar plates (11), and all experiments were performed in an anaerobic box (17). TSY broth contained 17 g of Trypticase (BBL Microbiology Systems, Cockeysville, Md.), 3 g of Phytone (BBL), 5 g of yeast extract (Difco Laboratories, Detroit, Mich.), 2.5 g of glucose, 5 g of NaCl, and 2.5 g of K_2HPO_4 per liter of distilled water. The broth was prepared in an anaerobic box and then autoclaved in tightly stoppered tubes. A defined medium (MOPS) was prepared as described by Neidhardt et al. (14). This medium was supplemented with thymine (100 μg/ml) for strains W3110 and p3478. All components (except water) were filter-sterilized in stock solutions under aerobic conditions. Anaerobic water was autoclaved in tightly capped bottles. The medium was then prepared in the anaerobic box and stored in the box for at least a week before use. Hydrogen peroxide (30% wt/wt; Perhydrol) was from E. Merck AG, Darmstadt, Germany. The HEPES dilution solution had the following composition: 130 mM NaCl, 2 mM $MgSO_4$, and 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)-KOH (pH 7.5).

The strain to be exposed to hydrogen peroxide was grown at 37°C in TSY broth overnight and then inoculated into the MOPS medium. When the culture was in exponential growth phase and had a density of 0.2 (absorbancy at 600 nm), it was diluted in the HEPES dilution solution at 28 ± 0.5°C to a density of about 2×10^3 organisms per ml. Three minutes after the start of the dilution procedure, the organisms were exposed to 200 μM hydrogen peroxide. Samples (0.1 ml) were taken at regular time intervals and spread over the surface of duplicate blood agar plates. The plates were incubated for 1 day at 37°C, and the numbers of surviving organisms after various times of exposure to hydrogen peroxide were determined. Each strain was tested three times, and the mean of these determinations is given. For determination of the ability of intact cells to decompose hydrogen peroxide, a sample (2 ml) of the culture in the MOPS medium was transferred to the chamber of an oxygen monitor (model 53; Yellow Springs Instrument Co., Yellow Springs, Ohio) containing 2 ml of anaerobic 3 mM hydrogen peroxide in HEPES dilution solution. The change of oxygen concentration in the reaction mixture was fol-

TABLE 1. *Killing of various DNA repair-deficient and proficient strains of E. coli K-12 by hydrogen peroxide and the levels of catalase and superoxide dismutase in cell-free extracts and the ability of intact cells to decompose hydrogen peroxide*

Strains and chromosomal markers	% of organisms killed after 20 min exposure to 200 μM H_2O_2	nmol H_2O_2 decomposed by 10^6 cells per min	Catalase activity (μmol H_2O_2 decomposed per min per mg of protein) (3)	Superoxide dismutase activity (U/mg of protein) (12)
DNA repair-proficient strains				
AB1157 wt[a]	0	2	4	19
W3110 wt[b]	29	3	102	45
DNA repair-deficient strains				
JC5495 *recA13 recB21*[a]	>99	3	5	23
JC5544 *recA13 recC22*[a]	>99	3	3	16
AB2480 *uvrA6 recA13*[a]	>99	7	5	17
JC5547 *recA13 recB21 recC22*[a]	97	3	4	15
JM12123 *recA13*, as *a* but from JM12	95	1	5	15
AB2463 *recA13*[a]	95	2	4	11
X7026 *recA13* Δ(*lac-proB*) *supE*	93	4	4	12
JC7516 *recA56 recB21*, as *a* but *phx*	90	3	10	15
D21 *recA13*[c]	87	8	12	15
GY5208 *lexB31*, as *a* but *ilvK633*	81	<0.1	2	7
AB2474 *lexA1 uvrA6*[a]	78	<0.1	4	12
AB2470 *recB21*[a]	57	2	4	19
SA291 Δ*uvrB*[d]	46	9	20	21
JC5519 *recB21 recC22*[a]	44	3	9	20
p3478 *polA1*[b]	30	5	9	62
AB3058 *recC22*, as *a* but *deoB16*	16	22	5	24
AB1885 *uvrB5*[a]	11	25	13	53
AB1884 *uvrC34*[a]	9	1	2	11
JM1253 *zab-53*, as *a* but from JM12	9	<0.1	5	14
AB1886 *uvrA6*[a]	0	2	3	22
JC7623 *recB21 recC22*[a]	0	2	<0.05	8
JC9239 *recF43*[a]	0	2	5	13
JM12 *tif-1*[a]	0	<0.1	2	13

[a] *thr-1 leuB6 thi-1 argE3 his-4 proA2 lacY1 galK2 ara-14 xyl-5 mtl-1 rpsL tsx-33 supE44* F^- (2).
[b] *thy deoC* F^-.
[c] *his proA trp lacY1 rpsL* Amp_{20} Tg $Öu^r$ Öus 51^r F^-.
[d] *his* Δ(*galK-attλ-bio-uvrB*) F^-.

lowed for 15 min, and the amount of oxygen evolved per minute was determined.

For preparation of cell-free extracts, the strains were grown at 37°C in TSY broth for 8 h and then in the MOPS medium overnight. The cultures were harvested by centrifugation at 20,000 × *g* for 15 min at 4°C and washed twice with HEPES dilution solution, and cell-free extracts were prepared (17). The activity of catalase in the cell-free extract was determined as described by Beers and Sizer (3). Superoxide dismutase activity was determined by the method of Marklund and Marklund (12). Peroxidase activity was assayed with guaiacol as hydrogen donor (4). The ability of intact cells to decompose hydrogen peroxide and the enzyme activities of cell-free extracts were tested in two independent experiments and, when there was a significant difference between the two determinations, the test was repeated.

When the strains were exposed to 200 μM hydrogen peroxide, *recA* strains were more rapidly killed than the other DNA repair-deficient strains (Table 1). There were surprisingly big consistent differences in levels of catalase and superoxide dismutase in cell-free extracts of the strains. Similar big differences were also found in the ability of intact cells of the strains to decompose hydrogen peroxide. The levels of peroxidase in the cell-free extracts were too low to be determined (less than 0.05 μmol of H_2O_2 consumed per min per mg of protein).

There was no correlation between the killing rate of the strains and their ability to decompose hydrogen peroxide by intact cells or the level of catalase and superoxide dismutase in cell-free extracts.

The enzymes catalase, peroxidase, and superoxide dismutase can be considered a primary defense of the cell against the dangerously re-

active products formed upon the cellular reduction of oxygen to water (8). The present study demonstrated that a functional *recA*$^+$ gene product is more important than these enzymes in protecting *E. coli* K-12 against the toxic effects of hydrogen peroxide. Hydrogen peroxide induces single-strand DNA breaks in *E. coli* and may also impair that repair of DNA, which is dependent on a *recA*$^+$ function (10). The *recA*$^+$ gene product has recently been purified and shown to catalyze in vitro the formation of duplex DNA from complementary single strands and to unwind DNA in the presence of ATP (7, 13, 15, 16). Such reactions are consistent with a direct role of the *recA* protein in genetic recombination and in the filling of single-strand gaps during postreplication repair. Studies on the functions of the *recA* protein in vitro open new possibilities for exploring the role of *recA* protein in hydrogen peroxide toxicity.

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