The Effect of Catalase on Recovery of Heat-injured DNA-repair Mutants of *Escherichia coli*

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The apparent sensitivity of *Escherichia coli* K12 to mild heat was increased by *recA* (*def*), *recB* and *polA*, but not by *uvrA*, *uvrB* or *recF* mutations. However, addition of catalase to the rich plating medium used to assess viability restored counts of heat-injured *recA*, *recB* and *polA* strains to wild-type levels. *E. coli* p3478 *polA* was sensitized by heat to a concentration of hydrogen peroxide similar to that measured in autoclaved recovery medium. The apparent heat sensitivity of DNA-repair mutants is thus due to heat-induced sensitivity to the low levels of peroxide present in rich recovery media. It is proposed that DNA damage in heated cells could occur indirectly by an oxidative mechanism. The increased peroxide sensitivity of heat-injured cells was not due to a decrease in total catalase activity but may be related specifically to inactivation of the inducible catalase/peroxidase (HPI).

INTRODUCTION

Exposure of microbes to mild heat results in damage to many cell components including membranes, nucleic acids, ribosomes and proteins, but the critical events leading to loss of viability remain poorly understood (Hurst, 1984). Several workers have suggested that DNA may be an important target of heat injury (reviewed by Pellon & Sinskey, 1984). Evidence supporting this idea is of three types: first, genetic loss of certain DNA-repair functions sensitizes *Escherichia coli* to heat (Bridges *et al.*, 1969*a, b*); second, mild heat produces single- and double-strand breaks in DNA which are repaired during subsequent incubation in buffer with coincident restoration of viability (Sedgwick & Bridges, 1972; Woodcock & Grigg, 1972); third, mild heat induces changes in the sedimentation coefficient of the bacterial nucleoid (Pellon & Sinskey, 1984).

Despite this evidence there remains uncertainty about the role of DNA damage in thermal inactivation and the mechanism by which strand breaks occur. Sedgwick & Bridges (1972) reported that the kinetics of strand breakage and their relation to loss of viability varied depending on the strain tested. They proposed that the mechanism of DNA strand breakage was indirect, possibly involving the action of a nuclease. Williams-Hill & Grecz (1983) subsequently showed that strains lacking an apurinic endonuclease were more resistant to mild heat and sustained fewer strand breaks in their DNA than the corresponding parent organisms.

The recovery of several species of bacteria following heat injury is improved by incorporating catalase in recovery media, implying that heated cells are sensitive to hydrogen peroxide (Baird-Parker & Davenport, 1965; Brewer *et al.*, 1977; Rayman *et al.*, 1978; Mackey & Derrick, 1982). Hydrogen peroxide causes several types of damage to DNA, therefore peroxide, or oxygen-containing radicals derived from it, could constitute an indirect mechanism of DNA damage in heated cells.

In this paper we examine the effects of catalase and hydrogen peroxide on the viability of DNA-repair mutants of *E. coli* injured by mild heating.

Table 1. *Strains used and their source*

Strain	Relevant genotype	Reference	Source*
AB1157	Parent	Howard-Flanders <i>et al.</i> (1966)	CGSC
AB1886	<i>uvrA6</i>		CGSC
AB1885	<i>uvrB5</i>		CGSC
AB2463	<i>recA13 (def)</i>	Howard-Flanders & Theriot (1966)	CGSC
AB2470	<i>recB21</i>	Howard-Flanders (1968)	B. Wilkins
AB2494	<i>lexA1(ind)</i>		CGSC
AB2474	<i>lexA1 uvrA6</i>		CGSC
AB2480	<i>recA13 uvrA6</i>	Howard-Flanders <i>et al.</i> (1969)	CGSC
JC9239	<i>recF143</i>	Horii & Clark (1973)	A. J. Clark
W3110	Parent, <i>thyA36</i>	De Lucia & Cairns (1969)	M. H. L. Green
p3478	<i>polA1 thyA36</i>		M. H. L. Green
KL16	Parent	White <i>et al.</i> (1976)	B. Weiss
BW9101	$\Delta(pncA-xthA)$		B. Weiss
CSH 7	Parent	Loewen (1984); Loewen <i>et al.</i> (1985)	S. Moss
UMI	<i>katE1 katG14</i>		S. Moss

* CGSC strains were received from B. Bachmann, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn., USA. Other addresses are: B. Wilkins, Department of Genetics, University of Leicester, Adrian Building, University Road, Leicester LE1 7RH, UK; A. J. Clark, Department of Molecular Biology, University of California, Berkeley, Calif. 94720, USA; M. H. L. Green, MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton BN1 9RR, UK; B. Weiss, Department of Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, Baltimore, Md 21205, USA; S. Moss, School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7HY, UK.

METHODS

Bacteria. Strains of *E. coli* K12 used in this work are listed in Table 1. The complete genotypes are described in the references listed in the table.

Media diluents and buffer. Tryptone soya broth (TSB Oxoid CM129) was supplemented with 0.3% (w/v) yeast extract and, where necessary, 20 μ g thymidine ml⁻¹. The corresponding agar (TSA) contained 1.2% (w/v) New Zealand agar. Where indicated, catalase (Sigma C40; 0.08 ml of a 2 mg ml⁻¹ solution containing 1760 units) was spread over the surface of agar plates and allowed to dry. M9 minimal medium contained, per litre: Na₂HPO₄, 6 g; KH₂PO₄, 3 g; NaCl, 0.5 g; NH₄Cl, 1 g; CaCl₂, 0.011 g; MgSO₄·7H₂O, 0.246 g; glucose, 2 g. Amino acids to satisfy auxotrophic requirement were added at 20 μ g ml⁻¹. Peptone saline contained per litre: Bacto peptone (Difco B118), 1 g; NaCl, 8.5 g; adjusted to pH 7.0. The phosphate buffer used in these experiments was 0.1 M-potassium phosphate pH 6.0 unless otherwise stated.

Growth of organisms and heat treatment. Cells were grown for 16–20 h at 37 °C in 10 ml TSB contained in a 50 ml conical flask in a water bath set at 100 r.p.m. Stationary phase culture (0.2 ml) was inoculated into 50 ml fresh medium in a 250 ml flask shaken at 300 r.p.m. When the OD₆₈₀ had reached 0.5, cells (11 ml) were harvested by filtration washed once and resuspended in 7 ml phosphate buffer. Washed cell suspension (0.3 ml) was placed in a freeze-drying ampoule (100 × 7 mm) which was then sealed in a gas flame. Sealed ampoules were immersed in a water bath at 52 °C for predetermined intervals, then removed and placed in ice.

Viable counts. Serial decimal dilutions were made in peptone saline and duplicate or quadruplicate 0.02 ml volumes, delivered from 20 SWG stainless steel cannulae, were spread on quarter sections of agar plates. Colonies were counted after 24 h incubation at 37 °C. Despite careful standardization of growth, harvesting and heating conditions, variations in survival curves were observed between replicate experiments, especially when these were done many months apart. The reason for this is not known but it may have been due to slight variations between different batches of diluent, media, etc. To take account of this variation, heat survival curves of mutants were compared with the wild-type strain tested on the same day and using the same batch of media and diluents.

The degree of variation was calculated for data shown in Fig. 1 in which each plotted point is the mean value from three independent experiments. Standard errors of the mean count increased with heating time and were greater on TSA than on TSA plus catalase. The range of standard errors were 0–0.43 and 0–0.53 log₁₀ units for counts on TSA and TSA plus catalase respectively. Survival curves shown in other figures are from single, representative experiments.

Peroxide sensitivity. *E. coli* p3478 was grown to OD₆₈₀ 0.5, harvested by filtration, then resuspended in twice the original volume of phosphate buffer. Portions (0.1 ml) of suspension were added to 9.9 ml M9 medium plus thymidine, with or without 25 μ M-H₂O₂, incubated at 37 °C. Samples (1 ml) were withdrawn at intervals and added to 9 ml peptone saline containing 1760 units catalase. Cells heated at 52 °C for 6 min in sealed ampoules were treated similarly. Viable counts were determined after appropriate dilution.

Enzymes. Catalase activity was measured by the method of Rorth & Jensen (1967) using a Clark oxygen electrode (Yellow Springs Instruments). Cells were grown to OD_{680} 0.5, harvested by centrifugation, then washed and resuspended in phosphate buffer to OD_{680} 10.0; 1 ml of this concentrated suspension was added to 9 ml buffer pre-heated to 54.5 °C. This raised the temperature of the suspension to 52 °C instantaneously. The tube containing the suspension was quickly transferred to a water bath at 52 °C and samples (1 ml) were removed at intervals thereafter for catalase determination. The reaction mixture contained 1 ml cell suspension, 2 ml phosphate buffer and 15 mM- H_2O_2 . Protein was measured by the method of Lowry. Catalase C-40, peroxidase P-8250 and superoxide dismutase S-8254, used in the protection experiment, were from Sigma.

DNA degradation. Cells were labelled by inoculating 0.04 ml of an 18–24 h culture in TSB plus 2 µg thymidine ml^{-1} into 5 ml fresh TSB containing 1 µg thymidine ml^{-1} plus [3H]thymidine (185 kBq ml^{-1} ; 5 µCi ml^{-1}) and incubating until the OD_{680} was about 0.5. Cells were harvested by filtration, washed with 6 vols phosphate buffer, then resuspended in 5 ml buffer. Samples (1 ml) were heated by adding to a test tube immersed in a water bath at 52 °C. After 6 min the tube was placed in ice and 0.2 ml volumes were added to 10 ml TSB or M9 plus thymidine (3 µg ml^{-1}) with and without catalase (1760 units per tube). Portions (0.15 ml) of suspension were removed at intervals and spotted onto filter paper (Whatman 3M). When dry the filter papers were washed twice in 5% (w/v) ice-cold trichloroacetic acid, and once each in 95% ethanol and acetone. The dry filters were counted in Instagel scintillation fluid (Packard Instrument Co.).

RESULTS

Effect of catalase on viable counts

Survival curves of strains of *E. coli* heated in phosphate buffer at 52 °C are shown in Figs 1 and 2. Data are normalized so that the initial (unheated) counts on TSA plus catalase equal 100% for all strains. When viability was compared with the wild-type on TSA, the *recA13* and *polA1* strains were apparently more heat sensitive whereas the *uvrA6* strain was of equal heat resistance (Fig. 1a). However, when catalase was present in the plating medium there were no significant differences between strains (Fig. 1b).

Fig. 1

Fig. 1. Survival of bacteria heated at 52 °C. Viable counts estimated on TSA (a) or TSA plus catalase (b). ●, Wild-type AB1157; ▲, wild-type W3110; ○, AB2463 *recA13*; △, W3110 *polA1*; □, AB1886 *uvrA6*.

Fig. 2

Fig. 2. Survival of bacteria heated at 52 °C. Viable counts estimated on TSA (●, ▲) or TSA plus catalase (○, △). ●, ○, Wild-type AB1157; ▲, △ AB2474 *uvrA6 lexA1*.

Fig. 3. Loss of viability of heat-injured *E. coli* p3478 *polA1* exposed to rich medium or hydrogen peroxide. (a) Bacteria heated at 52 °C for 6 min were inoculated into TSB at 37 °C containing: no addition (●), superoxide dismutase (○), catalase (▲), or peroxidase (△), (100 µg ml⁻¹ each). (b) cells heated at 52 °C for 6 min (▲, △) or unheated (●, ○) were inoculated into M9 medium at 37 °C (●, ▲) or M9 plus 25 µM hydrogen peroxide (○, △).

Mutations at *uvrB5* or *recF143* had little effect on survival whereas *recB21* was similar in its effect to *recA13* (data not shown). The effect of *lexA1* was more complex; on its own, *lexA1* had no effect on survival whereas the *lexA1 uvrA6* double mutant exhibited lower survival than the parent on TSA even in the initial unheated sample (Fig. 2). Similar survival curves were obtained with the *uvrA6 recA13* strain and, occasionally, with the *recB21* strain and an additional *recA1* strain (JC 1553). In all cases, however, counts were restored to wild-type levels by the addition of catalase to recovery media. Anaerobic incubation of TSA plates without catalase did not increase recoveries significantly.

Effect of catalase, peroxidase and superoxide dismutase on post-heating survival. The results described above imply that loss of viability of heat-injured cells occurred after heating, when cells were exposed to nutritionally rich growth medium. The kinetics of viability loss on rich medium were investigated in *E. coli* p3478, the most sensitive strain. Heat-injured cells suspended in TSB lost viability within 30 min (Fig. 3a). Catalase and peroxidase were protective but superoxide dismutase had only a marginal or (in some experiments) nil effect.

Sensitivity of heat-injured cells to peroxide. Protection against loss of viability by catalase implies (a) that heated cells are sensitive to peroxide and (b) that peroxide is present in rich growth medium. The concentration of peroxide in 10 samples of autoclaved TSB ranged from 12 to 30 µM with a mean of 20 µM. No peroxide was detected in M9 medium (Mackey & Derrick, 1986). Loss of viability of heat-injured *E. coli* p3478 in M9 plus 25 µM-hydrogen peroxide followed a similar course to that in TSB (Fig. 3b). Unheated cells remained viable under the same conditions.

Effect of heat on cellular catalase activity. Whole-cell catalase activity was about 85% of the level in cell extracts. Fig. 4(a) shows that whole-cell catalase activity in *E. coli* p3478 increased slightly during the first 3 min of heating, then declined over the next 27 min to 35% of the initial level. For comparison, the rate of increase in peroxide sensitivity (as indicated by the difference in viable count on TSA and TSA plus catalase) is shown on the same diagram (data taken from Fig 1a). Peroxide sensitivity evidently occurs before any substantial loss of total catalase activity.

E. coli contains two catalases (hydroperoxidases), designated HPI and HPII (Claiborne & Fridovich, 1979; Claiborne *et al.*, 1979; Loewen, 1984; Loewen *et al.*, 1985; Meir & Yagil, 1984, 1985). The HPI enzyme possesses catalase and peroxidase activity, is inducible and heat labile whereas HPII lacks peroxidase activity, is constitutive and relatively heat stable. To examine the effect of heat on cellular HPI activity, the experiment described above, using *E. coli* p3478,

Fig. 4. Inactivation of catalase during heating of *E. coli* at 52 °C. (a) ●, Whole-cell catalase activity of *E. coli* p3478 *polA1*; ○, catalase activity of cell extract of unheated cells; △, increase in peroxide-sensitivity measured as the difference in log₁₀ count on TSA and on TSA plus catalase (data recalculated from Fig. 1). (b) Inactivation of HPI during heating of *E. coli* UMI at 52 °C.

was repeated using *E. coli* UM1, which lacks HP11. Cells were grown in LB medium, which contains no glucose, rather than TSB, to ensure that HPI was present at reasonably high levels. In contrast to total catalase activity, HPI activity declined rapidly during heating at 52 °C, decreasing to 22% of its initial level within 3 min (Fig. 4b). Inactivation of total catalase in the parent strain CSH7, also grown in LB medium, followed a similar course to that of strain p3478 grown in TSB, although absolute activities were slightly higher throughout (data not shown). Heat-injured cells of strain UMI were no more sensitive to peroxide in plating media than cells of the parent strain CSH7 (data not shown).

Effect of the *xthA* mutation on heat resistance. Lack of exonuclease III did not affect heat-induced peroxide sensitivity as judged by the difference between counts on TSA and TSA plus catalase for the two strains. However, strain BW9101 was slightly more heat resistant than KL16 irrespective of the recovery medium used to assess viability. After 12 min heating at 52 °C, percentage survival was 4.3 and 0.57 for strains BW9101 and KL16 respectively with TSA plus catalase as recovery medium, and 0.54 and 0.35% with TSA minus catalase.

DNA breakdown in *E. coli* p3478. Heat-injured cells of *E. coli* p3478 degraded about 26–28% of their DNA over 2 h when incubated in TSB or M9 medium. Addition of catalase to the media reduced this to 9–10%. Similar degradation (about 20%) also occurred when cells were washed in phosphate buffer but not heated before adding to TSB. The extent of this degradation in unheated cells was reduced to about 12% if the washing buffer contained 10 mM-MgCl₂ (data not shown).

Repair of heat injury. When heat-injured cells of *E. coli* p3478 were incubated in M9 medium, colony counts on TSA increased to equal those on TSA plus catalase, i.e. cells recovered the ability to grow on peroxide-containing medium (Fig. 5a). By contrast, colony counts on TSA plus catalase decreased to the level on TSA when cells were incubated in TSB, i.e. peroxide-sensitive cells died. Similar results were obtained with AB2463 *recA13* (data not shown). The wild-type AB1157 repaired injury in M9 or TSB, although repair took longer in TSB (Fig. 5b). (Note that AB1157 was heated for 12 min rather than 6 min to obtain a sufficient difference between counts on the two media for repair to be measurable.) The decline in viability in M9 subsequent to repair was somewhat variable and was not investigated further. Strain AB2494 *lexA1* repaired in M9 but showed simultaneous repair and loss of viability in TSB (data not shown). Repair in M9 thus occurred in wild-type, *lexA1*, *recA13* and *polA1* strains, but only the wild-type and the *lexA1* strain (partially) could repair in TSB.

Fig. 5. Repair of heat injury in (a) *E. coli* p3478 *polA* and (b) *E. coli* AB1157 wild-type. Cells were heated in phosphate buffer at 52 °C for (a) 6 min or (b) 12 min, then inoculated into M9 (●, ○) or TSB (■, □). Viable counts were determined at intervals on TSA (○, □) or TSA plus catalase (●, ■).

DISCUSSION

In agreement with previous workers we have found that mutants of *E. coli* lacking certain DNA-repair functions are more sensitive to mild heat than wild-type strains (Bridges *et al.*, 1969*a,b*; Pauling & Beck, 1975; Ahmad *et al.*, 1978). However, this thermosensitivity is only apparent, and results from a heat-induced sensitivity to the low levels of peroxide present in rich media used to assess viability. Heat-injured DNA-repair mutants are more peroxide-sensitive than repair-competent strains and therefore form fewer colonies when plated on rich medium.

Mutations having the greatest effect on apparent heat sensitivity were, in order of increasing effect: *lexA1*, *recA13* or *recB21* and *polA1*. Strains carrying *uvrA6*, *uvrB5* or *recF143* mutations were of similar resistance to the wild-type. The effect of DNA-repair mutations on heat resistance has been examined previously by others (Bridges *et al.*, 1969*a,b*; Sedgwick & Bridges, 1972; Ahmad *et al.*, 1978). There is agreement that the *recA13* mutation sensitizes cells to heat whereas *uvrA* or *uvrB* mutations are without effect. There are differences in the reported effects of other mutations. Both we and Ahmad *et al.* (1978) found *E. coli* p3478 *polA1* to be much more heat sensitive than the parent W3110 whereas Sedgwick & Bridges (1972) found no difference between these two strains but did find *E. coli* R15 *res* to be more heat sensitive than the parent H/r 30-R (the *res* allele in *E. coli* B strains is analogous to *polA* in K12 strains). The effect of the *lexA1* mutation on apparent heat resistance is difficult to interpret; on its own, *lexA1* had little effect, but the *lexA1 uvrA6* strain was sensitive to rich medium even without prior heating and that sensitivity was not much increased by subsequent heating. This effect was sometimes observed with AB2480 *uvrA6 recA13*, JC1553 *recA1* and, more rarely, AB2470 *recB21*. Cold shock or diluent stress can induce sensitivity to peroxide (Mackey & Derrick, 1986) and we suspect that the strains described above are particularly sensitive to the washing procedures used in preparing cell suspensions.

If LB agar, which contains less peroxide, was substituted for TSA, the initial (unheated) counts of strain AB2474 *lexA1 uvrA6* on media with and without catalase were similar, but sensitivity to LB agar (minus catalase) developed during heating (data not shown), i.e. when LB agar was used as recovery medium AB2474 *lexA1 uvrA6* displayed apparent heat sensitivity. Our results are thus somewhat similar to those of Bridges *et al.* (1969*a*) who found that *lexA* (*ind*) had little effect on heat resistance unless combined with *uvr* or *lon* mutations. In contrast, Ahmad *et al.* (1978) found the *lexA1* strain to be as heat sensitive as the *recA13* strain and further differed from us in finding no effect of *recB21*.

The reason for the discrepant results with *polA1* and other strains is not clear at present but may be related to different levels of peroxide in media used to assess viability and also to differences in diluents used for washing and heating cells which may affect membrane integrity (see below).

Survival curves of heated cells were often triphasic, containing initial and final steep portions and a central plateau (e.g. see strains AB1157, AB1886 and W3110 in Fig. 1a). This was reproducible and has been reported previously by Grecz *et al.* (1985), who ascribed the effect to heat-shock-induced thermotolerance. We have not investigated the phenomenon further. The non-linear kinetics preclude resistances being compared on the basis of the gradients of the slopes of the survival curves.

If the apparent heat sensitivity of DNA-repair mutants is due to their greater sensitivity to peroxide in recovery media, one would expect there to be a correlation between sensitivity to heat and to authentic peroxide. The peroxide sensitivity of the relevant DNA-repair-deficient strains has been examined by several workers (Carlsson & Carpenter, 1980; Ananthaswamy & Eisenstark, 1977; Imlay & Linn, 1986). There is agreement that the *recA* (*def*) mutation sensitizes cells to peroxide whereas *uvrA* and *uvrB* mutations do not. This corresponds exactly with the effect of these mutations on heat resistance. As with sensitivity to heat there are differing reports on the effect of the *polA* mutation on peroxide sensitivity. Ananthaswamy & Eisenstark (1977) and Imlay & Linn (1986) found that *polA* strains were more sensitive than wild-type strains to 10 mM- and up to 3 mM-H₂O₂ respectively, whereas Carlsson & Carpenter (1980) found no increased sensitivity of a *polA* strain to 0.2 mM-H₂O₂. Our results and those of Imlay & Linn (1986) showed *recB* strains to be more peroxide sensitive than the wild-type whereas Ananthaswamy & Eisenstark found that *recB* had a negligible effect.

It has recently become apparent that the response of cells to challenge by hydrogen peroxide is highly dependent on the growth conditions of the cells before the challenge, the temperature and composition of any diluents used to wash the cells, and the concentration of peroxide to which the cells are exposed (Mackey & Derrick, 1986; Imlay & Linn, 1986). It is thus likely that differences in experimental conditions could account for discrepant results reported by different workers.

However, despite individual differences, one can conclude that DNA-repair mutations that sensitize cells to hydrogen peroxide also cause increased (apparent) sensitivity to heat (the *xth* mutation is an exception which will be discussed below).

The mechanism by which peroxide causes cell-death is not clear at present. The simplest explanation is that, in heat-injured cells, peroxide causes damage to DNA which is inefficiently repaired in *polA*, *recA* (*def*), *recB* and possibly *lexA* (*ind*) strains. The *uvrA*, *uvrB* and *recF* functions are apparently less important in repairing this type of damage. The nature of the DNA damage is unknown but could be any of a variety of lesions caused by peroxide, including strand breakages, depurination and addition reactions with the double bond of bases (Yamafuji & Uchida, 1966; Rhaese & Freese, 1968; Demple & Linn, 1982). Strand breaks in DNA were detected when heated cells of *Salmonella typhimurium* were exposed to rich medium (Gomez & Sinskey, 1973). Our results suggest that oxidative damage could have been involved in this phenomenon.

The *xthA* gene codes for exonuclease III, a multifunctional enzyme possessing 3' → 5' exonuclease, apurinic endonuclease, phosphatase and ribonuclease H activities (Weiss, 1981). Cells lacking the enzyme are hypersensitive to hydrogen peroxide (Demple *et al.*, 1983) but the heat-injured *xthA* strain was no more sensitive than the wild-type to the low levels of peroxide in rich media. This could suggest that DNA damage caused by exposing unheated cells to reagent hydrogen peroxide differs from that caused by exposing heat-injured cells to complex media. Both types of lesion appear to require *polA*⁺ and *recA*⁺ functions whereas only the former requires *xthA*⁺. However, this conclusion is complicated by the fact that the normal physiological functions of exonuclease III are disturbed in heat-injured cells. Williams-Hill & Grecz (1983) showed that *E. coli xth* strains lacking exonuclease III sustained fewer DNA strand breaks during heating and survived better than otherwise isogenic *xthA*⁺ strains. They suggested that mild heating triggered exonuclease III activity leading to DNA damage and death of the cell. In our experiments catalase improved the recovery of both *xthA* and wild-type organisms, implying that exonuclease III activity is not required for peroxide-induced loss of viability in heat-injured cells and, conversely, that exposure to peroxide in rich media is not necessary for exonuclease III-mediated cell death.

Gross degradation of DNA occurs in *polA* strains of *E. coli* following DNA damage caused by ultraviolet or ionizing radiation and is believed to contribute to the radiation sensitivity of these strains (Boyle *et al.*, 1970). When heat-injured *E. coli* p3478 *polA* was resuspended in TSB, loss of viability and degradation of DNA occurred, both of which were reversed by catalase. However DNA degradation seems unlikely to account for loss of viability because the kinetics of the two processes were very different and heated cells resuspended in M9 medium degraded similar amounts of DNA with no loss of viability.

The increased sensitivity of heat-injured cells to low levels of peroxide in rich media is not due to a decrease in total catalase activity but may be related specifically to the inactivation or loss of catalase HPI. *E. coli* UMI lacks the major constitutive catalase (HPII) and reportedly contains only the peroxidatic activity associated with the minor, inducible catalase, HPI (Loewen *et al.*, 1985). In our hands strain UMI contained measurable residual catalase activity; because of its heat sensitivity, we believe this activity most likely to be associated with HPI (Meir & Yagil, 1985). The decrease in HPI activity that occurred under our heating conditions was sufficiently rapid to account for the correspondingly rapid increase in peroxide sensitivity. The K_m for H_2O_2 of HPI (about 3.7 mM) is much lower than that of the more thermostable HPII (reported as 18.2 or 30.9 mM) (Claiborne & Fridovich, 1979; Loewen & Triggs, 1984; Meir & Yagil, 1985) and is consistent with a protective function against low concentrations of peroxide. Chilling exponentially growing cells of *S. typhimurium* or *E. coli* also induces sensitivity to peroxide (Mackey & Derrick, 1986). These procedures and heating both affect membrane integrity and it is thus possible that membrane damage contributes to the enhanced sensitivity to peroxide.

When heat-injured cells were resuspended in minimal medium, colony counts on TSA increased to the level found on TSA plus catalase, i.e. cells recovered ability to grow on peroxide-containing media. We believe, therefore, that recovery of peroxide resistance accounts for the liquid holding recovery of heat-injured cells reported by others (Hershey, 1939; Mukherjee & Bhattacharjee, 1970; Stiles *et al.*, 1973; Gomez *et al.*, 1973; Ahmad *et al.*, 1978). The recovery process was independent of *lexA*, *recA* or *polA* and probably reflects repair or resynthesis of structures other than DNA, possibly HPI and the cell membrane. The process therefore differs from the 'minimal medium recovery' of ultraviolet-irradiated cells, which requires a functional *recA* gene (Sharma *et al.*, 1982).

Ahmad *et al.* (1978) found that the extent of liquid holding recovery of heated *E. coli* depended on the composition of growth and recovery media: when cells were grown in rich medium, heated then resuspended in minimal medium, no recovery took place within 2 h in wild-type, *lexA* (*ind*), *recA* or *polA* strains. We cannot explain why their results differ from ours but differences in the severity of heat treatment could be important.

To conclude, we support the notion that DNA damage caused by mild heating can occur indirectly and propose oxidative attack to DNA as a possible mechanism additional to the previously suggested endonucleolytic attack. The reason why heated cells are sensitive to peroxide and the nature of damage to DNA remain to be clarified.

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