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Glutathione in *Escherichia coli* Is Dispensable for Resistance to H_2O_2 and Gamma Radiation

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***Escherichia coli* devoid of glutathione (because of transposon insertions in the *gshA* gene) has normal resistance to H_2O_2, cumene hydroperoxide, heat, or ionizing radiation. Intracellular glutathione thus does not protect *E. coli* from such lethal oxidative damage. The use of *gshA*::Tn*10* mutants also revealed a glutathione-independent, H_2O_2-inducible resistance to *N*-ethylmaleimide.**

The tripeptide glutathione (L-γ-glutamyl-L-cysteinyl-glycine) (GSH) is the predominant low-molecular-weight thiol in many organisms and has been proposed to protect cells from oxidative damage such as that formed by H_2O_2 and ionizing radiations (19). *Escherichia coli* deficient in GSH because of mutations in the *gshA* gene was found previously (3) to exhibit normal resistance to X irradiation in the presence of oxygen. Other workers reported (21) that *E. coli gsh* mutants lacked the protective effect of hypoxia during γ irradiation. At least one of the mutants employed previously contains a second mutation that affects its sensitivity to H_2O_2 (10), probably because it was mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG; 3). To avoid this problem, we isolated transposon insertions in the *gshA* gene. Strains bearing a *gshA*::Tn*10* allele exhibit the expected hypersensitivities to thiol-specific reagents but are not at all defective in their resistance to oxidative damage, including γ rays.

The *E. coli* K-12 strains used were as follows: W3110 (*gsh*$^+$) and NK5991 (W3110 *srlD*::Tn*10*) supplied by N. Kleckner; AB1157 (*gsh*$^+$) and BS53 (AB1157 *gsh*) from B. Sedgwick (24); TJ5 (AB1157 *gshB*) supplied by J. Fuchs; and KL164 (*nalB14*) from the Coli Genetic Stock Center (5). Strain W3110 was mutagenized by the lambda hop procedure (26) with a derivative of Tn*10* (element 9 of Way et al. [26], hereafter called Tn*10*km) that confers kanamycin resistance (Kan^r). Colonies were collected from kanamycin (50 μg/ml) selection plates and stored at −50°C in 25% glycerol. Thawed cells were centrifuged and suspended in M medium (M9 salts [20] with 0.1 mM $CaCl_2$, 1.0 mM $MgSO_4$, 1 μg of thiamine hydrochloride per ml, 0.025% Casamino Acids, and 0.1% glucose) and enriched for *gshA* mutants essentially by the procedure of Sedgwick and Robins (24) by using one 45-min and two 10-min treatments with 50 μg of MNNG per ml. Most (70%) of the isolates obtained in this way showed substantially increased resistance to MNNG when tested individually. Of the three randomly selected MNNG-resistant isolates examined, all contained drastically diminished acid-soluble thiols (17). A putative *gshA*::Tn*10*km mutation was transduced from one of these isolates (JTG1) into AB1157 by using bacteriophage P1 (20) and selection for Kan^r. One Kan^r transductant (JTG10) was selected at random for further study; the mutation it bears was designated *gshA20*::Tn*10*km.

Cellular levels of reduced thiols are given in Table 1. These were quantitated by treating extracts (14) of thawed cells with *N*-[^{3}H]ethylmaleimide ([^{3}H]NEM; 53 Ci/mmol; New England Nuclear Corp.), followed by chromatography on silica thin-layer chromatography plates or Whatman no. 1 filter paper (15) in butanol-concentrated formic acid-H_2O (7:2:1, vol/vol/vol), localization of markers with ninhydrin, fractionation, and scintillation counting. JTG10 had <0.4% of the wild-type GSH level, compared to 1.4% residual GSH in strain BS53. In JTG10 and BS53 but not in the *gshB* mutant TJ5 (which accumulates the GSH precursor γ-glutamyl-cysteine), the decreased GSH levels appeared to be accompanied by lower amounts of cysteine in the cells. This apparent deficiency could be reversed by preventing oxidation with the inclusion of GSH before cell lysis.

Transduction of the *gshA20*::Tn*10*km mutation into NK5991 or KL164 gave cotransduction frequencies (18% with *srlD*; 34% with *nalB*) in agreement with the known map position of the *gshA* gene near 58 min (4, 5). Consistent with the *gshA* mutant genotype was the observation that JTG10 was exceptionally resistant to toxic agents thought to require thiols for their activation. This mutant was 10-fold more resistant than its parent (AB1157) to a challenge with MNNG (Fig. 1A) and 20-fold more resistant to a challenge with neocarzinostatin chromophore (22; Fig. 1B). The MNNG resistance of JTG10 was similar to that previously observed with BS53 (24). Treatment of mammalian cells with GSH-depleting agents has been reported to confer resistance to the toxic and mutagenic effects of neocarzinostatin chromophore (9).

TABLE 1. Quantitation of reduced GSH and cysteine contents in various strains

Strain	GSH added during extraction[a]	Acid-soluble thiols (μmol/10^{12} cells)		
		Total[b]	GSH[c]	Cysteine[c]
AB1157	−	5.5	5.1	0.39
JTG10	−	<0.1	<0.02	<0.01
	+	NM[d]	5.7	0.23
BS53	−	<0.1	0.07	<0.01
	+	NM	8.2	0.32
TJ5	−	6.7[e]	<0.02	0.40

[a] GSH added approximately to the wild-type level.
[b] Assayed according to the method of Lawley and Thatcher (17).
[c] Apparent breakdown products that accumulate upon storage of [^{3}H]NEM migrated near GSH and cysteine and so prevented assigning even lower limits on the amounts of these compounds detectable in the extracts of *gsh* mutant strains.
[d] NM, Not measured.
[e] This strain contained 6.0 μmol of γ-glutamyl-cysteine per 10^{12} cells.

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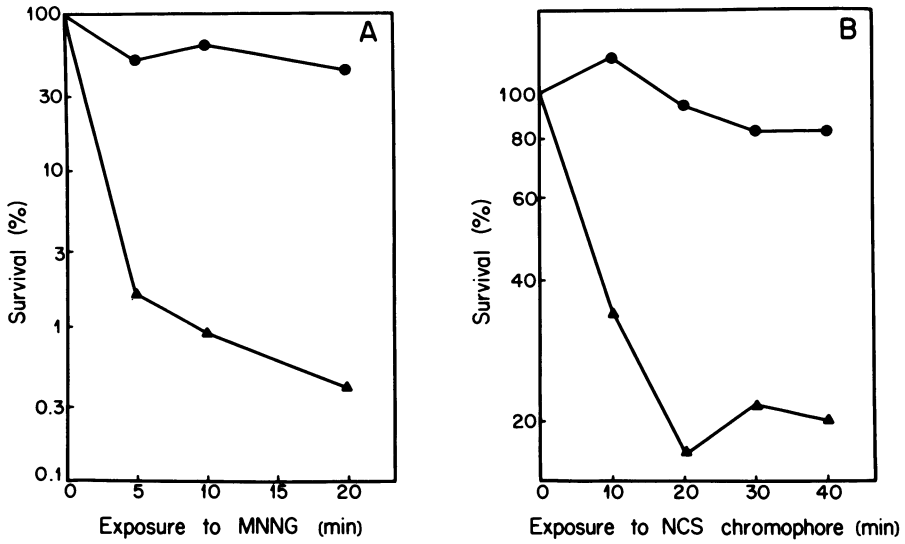

FIG. 1. Sensitivity to chemical mutagens. Exponentially growing strains cultured in M medium with 0.1% Casamino Acids were challenged at 37°C, and survival was determined by diluting cells in M9 salts and plating them on LB agar (20). Challenges: (A) 50 μg of MNNG per ml; (B) 0.22 μM neocarzinostatin (NCS) chromophore in the dark. Symbols: ▲, AB1157 (*gsh*$^+$); ●, JTG10 (*gshA20*::Tn*10*km).

In contrast to the suggestion that GSH protects cells from oxidative damage (see the review by Meister and Anderson [19]), strains JTG10 and BS53 were at least as resistant as AB1157 to 5 to 25 mM H_2O_2 challenges for up to 45 min (data not shown). JTG10 was also not especially sensitive to cumene hydroperoxide (data not shown). Bacteria lacking exonuclease III (12) and catalase (18) were not further sensitized to H_2O_2 by introduction of the *gshA20*::Tn*10*km mutation (data not shown). Thus, possible protection by GSH is not masked by other protective or repair activities. *E. coli* and *Salmonella typhimurium* possess an adaptive response to oxidizing agents which is triggered by low levels of H_2O_2 and which increases the survival of bacteria after toxic challenges with a variety of oxidative and other agents (7, 11). Strain JTG10 retained the normal H_2O_2-inducible resistance to H_2O_2, cumene hydroperoxide, and a 52°C heat challenge (data not shown). The acid-soluble thiol levels did not change in wild-type or *gsh* mutant *E. coli* during adaptation by H_2O_2.

The use of a *gshA* mutant reveals a novel feature of the adaptive response to oxidative damage (Fig. 2). Although unadapted JTG10 is sixfold more sensitive than AB1157 to the thiol reagent NEM, both strains show a fourfold increased resistance to NEM induced by H_2O_2. This induced resistance could reflect a change in the susceptibility to NEM alkylation (e.g., reduced membrane permeability) or a GSH-independent defense against alkylated thiols.

Ionizing radiations (X and γ rays) produce DNA damage via many of the same activated-oxygen species as H_2O_2 (23). The presence of oxygen enhances the toxicity of ionizing radiations severalfold (the oxygen enhancement ratio; 2, 16). To examine the effect of GSH on the oxygen enhancement ratio, bacteria were irradiated in the presence or absence of O_2 and assayed for survival (Fig. 3). The absence of GSH did not sensitize cells to killing by γ rays under either condition. JTG10 and AB1157 exhibited virtually the same oxygen enhancement ratio (3.7 and 3.9, respectively). This result conflicts with a previous report that GSH is responsible for protection by anoxia in *E. coli* (21). Evidently, the strain used in those experiments was unrelated to its *gsh*$^+$ parent (M. L. Morse, personal communication).

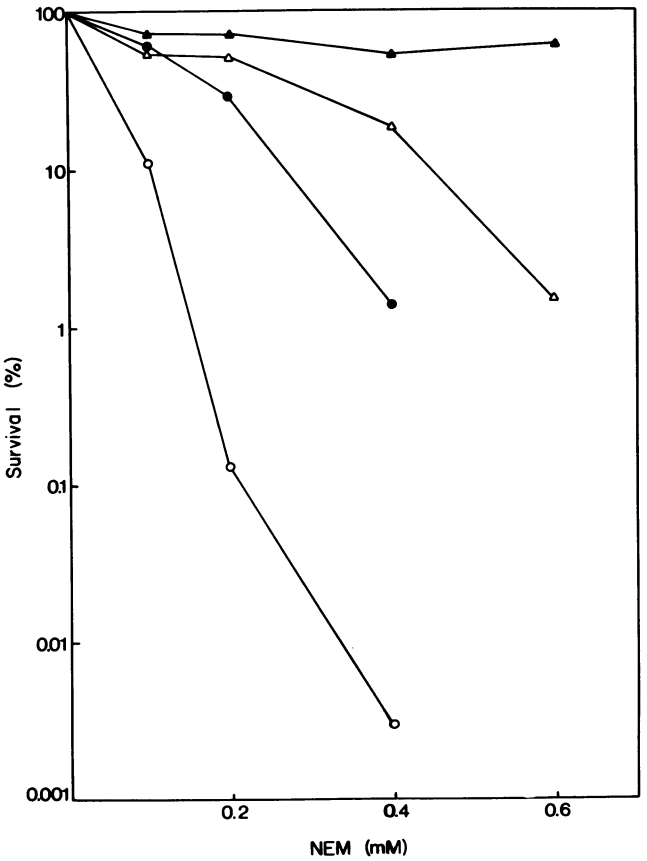

FIG. 2. Induction of NEM resistance by H_2O_2. Exponentially growing strains were pretreated as indicated with 56 μM H_2O_2 for 35 min and challenged with NEM for 10 min. Data shown are averaged from four (AB1157) or three (JTG10) experiments. Symbols: △,▲, AB1157 (*gshA*$^+$); ○,●, JTG10 (*gshA20*::Tn*10*km). Open symbols: not pretreated; filled symbols: pretreated with H_2O_2.

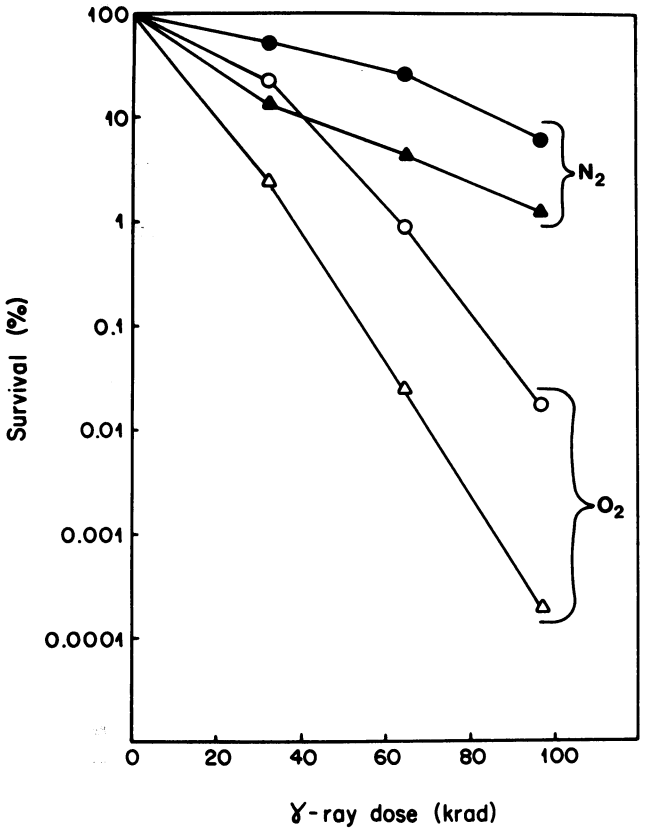

FIG 3. Effect of O_2 on γ radiation sensitivity. Bacteria growing in LB broth (20) were centrifuged, suspended in an equal volume of M9 salts, and irradiated at room temperature with a ^{60}Co source (2,130 rads/min; Laboratory of Radiobiology, Harvard School of Public Health, Boston, Mass.) under bubbling with purified N_2 (O_2 at 150 ppm during irradiation) or USP grade O_2. After irradiation, the cells were chilled on ice and diluted for plating. Data shown are averaged from two experiments. Symbols: ▲, AB1157 (N_2); △, AB1157 (O_2); ●, JTG10 (N_2); ○, JTG10 (O_2).

Bacteria lacking acid-soluble thiols (JTG10 and BS53) were slightly more resistant to γ rays or H_2O_2 (Fig. 3; data not shown) than were thiol-containing cells (AB1157 and TJ5). Perhaps the lethal effects of activated oxygen are potentiated by oxidized GSH or γ-glutamyl-cysteine, in a manner analogous to molecular oxygen (1, 2). Alternatively, since JTG10 does exhibit a slight growth disadvantage compared with AB1157 (unpublished data), the small extra resistance might be due to the slightly longer generation time in these mutants.

The data presented here indicate that GSH does not protect *E. coli* from oxidative damage. There are some indications that diminished GSH levels render mammalian cells more sensitive to hypoxic irradiation (6, 13). The role of GSH in bacteria thus may differ from its function in mammalian cells. One difference is that *E. coli* apparently lacks the enzyme GSH peroxidase (25), which in eucaryotes could provide GSH-dependent protection from oxidative damage. This is an open question because GSH depletion of mammalian cells had no effect in other experiments (8).

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