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Catalase has only a minor role in protection against near-ultraviolet radiation damage in bacteria

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Summary. In bacterial cells near-ultraviolet radiation (NUV) generates H_2O_2 which can be decomposed by endogenous catalase to H_2O and O_2. To assess the roles of H_2O_2 and catalase in NUV lethality, we manipulated the amount of intracellular catalase (a) by the use of mutant and plasmid strains with altered endogenous catalase, (b) physiologically, by the addition of glucose, and (c) by induction of catalase synthesis with oxidizing agents. Not only was there no direct correlation between NUV-resistance and catalase activity, but in some cases the correlation was inverse. Also, while there was correlation between NUV and H_2O_2 sensitivity for most strains tested, there were a number of exceptions which indicates that the modes of killing were different for the two agents.

Key words: Catalase – Peroxide – Near-ultraviolet radiation

Introduction

In our early studies on the effect of near ultraviolet radiation (NUV) on bacteria, we found that an important photoproduct of NUV was H_2O_2 which, in part, might have a role in NUV lethality (Ananthaswamy and Eisenstark 1976; McCormick et al. 1976). Since then, numerous observations have been made of the similarities between H_2O_2 and NUV effects (Ahmad 1981; Eisenstark 1985; Hartman 1986; Hartman et al. 1979; Sammartano and Tuveson 1983, 1985; Tyrrell 1985), consistent with the view that biologically relevant quantities of H_2O_2 may be generated in situ upon NUV irradiation of cells (Hartman 1986). However, it was also observed that certain DNA repair mutants with normal catalase concentration (e.g., *recA*, *xthA* and *polA*) were very sensitive to H_2O_2 and NUV (Ananthaswamy and Eisenstark 1976; Carlsson and Carpenter 1980; Sammartano and Tuveson 1983).

The availability of *kat* (catalase) mutants and plasmid-carrying strains led us to re-examine the relative importance of catalase in the recovery from NUV and H_2O_2 stress, and to question whether NUV and H_2O_2 lethal rates follow similar patterns.

Materials and methods

NUV and FUV survival curves. *Escherichia coli* and *Salmonella typhimurium* strains used are listed in Table 1. For inactivation studies (Fig. 1, log-phase cells growing in nutrient broth (NB) were centrifuged, the pellet was resuspended in M9 at a concentration of $1–5 \times 10^8$ cells/ml before NUV and FUV (for UV) exposure. Cell suspensions were irradiated by NUV in pyrex vessels covered by a sheet of cellulose acetate to filter wavelengths below 290 nm. For FUV inactivation curves, cells in open glass petri dishes were irradiated with a germicidal lamp. Irradiated samples were appropriately diluted and plated on nutrient agar.

H_2O_2 survival curves. Curves were obtained in two ways: (a) by varying the time of exposure to H_2O_2 (Table 1), and (b) by varying the H_2O_2 concentration and exposing cells for 10 min (Fig. 2). All strains were grown to about 10^8 cells/ml in NB and harvested by centrifugation at 4° C. The cells were washed and resuspended in M9 containing H_2O_2 (final concentration 16 mM), and incubated at 30° C. At various time intervals, samples were withdrawn, diluted, plated in duplicate on nutrient agar. Plates were incubated at 37° C for colony formation. Data for percent survivors after 20 min exposure are presented in Table 1. Survival curves were also determined by exposing cells for a fixed time (10 min) but at different H_2O_2 concentrations (Fig. 2).

Breakdown of H_2O_2 was assayed by two different methods: (a) Use of Clark O_2-electrode (Rørth and Jensen 1967). In this method H_2O_2 was added to 3 ml of cells (2.7 mM final concentration) and the rate of conversion to O_2 was measured (Table 1). (b) Colorimetric method of Sinha (1972) in which dichromate was reduced to chromic acid in the presence of H_2O_2. Measurements were made of the amount of H_2O_2 remaining after cells were allowed to react with 3.6 mM (final concentration) H_2O_2 for specific periods of time. The methods for induction of resistance to NUV and H_2O_2 were the same as previously described (Demple and Halbrook 1983; Peters and Jagger 1981; Sammartano and Tuveson 1985; Tyrrell 1985).

Results

Relation of catalase activity to NUV and/or H_2O_2 sensitivity in mutant and plasmid-carrying strains

If catalase were a key enzyme in protecting cells against NUV, it would be expected that *kat* mutants would be very sensitive to NUV. This is the case for *E. coli katF* and *S. typhimurium kat* mutants, which are very sensitive, but not for *E. coli katE* and *katG* which show only mild

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Table 1. Comparison of catalase content with NUV and H_2O_2 sensitivity

Strain, main phenotype and source[a]	Catalase[b] μmoles O_2/ml per hour per OD unit protein		Sensitivity (% Survival)			
			NUV fluence 3 Jm^{-2} (10^5)		H_2O_2 16 mM, 20 min	
	−G	+G	−G	+G	−G	+G
BW9109, *xthA* (White et al. 1976)	25	14	0.9	2.8	2.8	4.2
UM2, *katE katG* (Loewen and Triggs 1984)	6	4	1.6	23	3.0	18
UM56, *katF katG* (Loewen and Triggs 1984)	0	0	0.6	2.6	0.3	6.2
AB2463, *recA* (our laboratory)	19	9	0.03	1.2	0.01	0.06
polA546 (Chase & Masker 1972)	28	22	0.02	3.8	0.1	1.9
AB1157 wild-type for repair (our laboratory)	24	4	8.1	53	12	23
TA3303, *cpd401 cya408* (Alper and Ames 1975)	23	6	4.6	31	13	33
UM4, *katG* (Loewen and Triggs 1984)	2	2	2.6	18	6.0	12.1
UM120, *katE*::Tn*10* (Loewen and Triggs 1984)	1.9	2	4.5	20	8	20.0
6M122, *katF*::Tn*10* (Loewen and Triggs 1984)	3.1	3	0.2	2.9	0.3	2.2
UM181 *katE* (Loewen and Triggs 1984)	17	9	2.1	27	6	8
UM202 *katG*::Tn*10* (Loewen and Triggs 1984)	4.8	2.0	2.4	12.0	12	23.1
RT1 *nur* (Tuveson and Jonas 1979)	12	2	0.6	6.1	0.5	8.5
TC60, *katA1* (Levine 1977)	0	0	0.3	4.3	0.04	1.8
TC62, *katB2* (Levine 1977)	0	0	0.1	2.8	0.07	5.2
TC67, *katC6* (Levine 1977)	0.1	0	0.2	1.7	0.01	3.0
TA4112 *oxy Δ3* (Christman et al. 1985)	27	12	6.1	12	0.004	N.T.
TA4110 *oxyR* (Chrisman et al. 1985)	80	42	12	59	61	98
pep NABPQ (Strauch et al. 1985)	27	6	9.2	48	11	24
UM228 *kat recA* (Loewen et al. 1983)[c]	5.2	3.2	1.0	N.T.	0.2	N.T.
Same as UM228, with *katG*$^+$ in pBT22 (Loewen et al. 1983)	27	6.1	0.09	N.T.	2.2	N.T.

+G, with 0.5% glucose; −G, without glucose; N.T., Not tested

Wildtype alleles of most of the mutants were also tested, but are not included in this table. Results were similar to those obtained with AB1157. A number of additional *cya*, *cpd* and *crp* mutants were also tested. All of these showed glucose repression. Since cAMP is needed in "catabolite repression", this demonstrates that the observed glucose effect does not depend on cAMP

[a] TA3303, TC60, 62, 67 and pep NABPQ are *Salmonella typhimurium* strains, the remainder are *Escherichia coli* strains

[b] Catalase values were determined using a Clark oxygen electrode. They were also measured spectrophotometrically. A major difference between the two methods was that there was a larger difference between *oxyR* and its wildtype allele in the spectrophotometric method. Each OD unit equals 0.12 mg protein

[c] The *kat* defect in UM228 has not been completely characterized. It is not completely deficient in *katG* and is also either *katE* or *katF* (P. Loewen, personal communication). It produces a small amount of catalase

Dosage is expressed as joules per square meter. Numbers indicate percent survival at 3×10^5 joules per square meter

sensitivity (Table 1, Figs. 1 and 2). However, a number of mutants with normal catalase concentration are much more sensitive than *kat* mutants. The DNA metabolizing genes such as *recA, polA* and *xthA* are obviously more important than *kat* in protecting cells against oxidative damage (Christman et al. 1985).

Not only is there a lack of correlation between sensitivity and catalase contents among the various mutants, but there is not an absolute correlation between NUV and H_2O_2 sensitivity (Table 1). A striking example of this lack of correlation between catalase content and NUV sensitivity is in the comparison of strain UM228 with the same strain with a *kat* G^+ plasmid (Loewen et al. 1983; Table 1, Fig. 3). The plasmid-carrying strain contains considerably more catalase than does the strain without plasmid and is far more resistant to H_2O_2 (Table 1). However, it is much more sensitive to NUV than the non-plasmid allelic strain (Fig. 3).

As another example, *oxyA3* is hypersensitive to H_2O_2 but only slightly sensitive to NUV and it has normal catalase activity. The results with the *oxyR* mutant should also be noted as *oxyR* constitutively synthesizes increased catalase (Table 1; Christman et al. 1985), it would be expected to be resistant to both H_2O_2 and NUV. Indeed, it is resistant to H_2O_2, but shows comparatively less increased resistance to NUV (compare Fig. 1 with Fig. 2). Table 1 also presents results of inactivation of *nur*, a NUV-sensitive strain (Tuveson and Jonas 1979), the phenotypic defect of which has not yet been identified. However, its inactivation and catalase properties resemble that of *katF* (Loewen and Triggs 1984).

These differences support an earlier idea that NUV damage and recovery involve mechanisms in addition to those involving H_2O_2 alone. This idea is based in part on previous studies in which cells were irradiated with NUV in the prsence of H_2O_2 (Ahmad 1981; Ananthaswamy and Eisenstark 1976; Hartman et al. 1979; Hartman and Eisenstark 1978). If the effects of the two were in the same pathway they would be expected to be additive and not synergistic.

Repression of catalase activity by glucose

Table 1 demonstrates that cells grown in the presence of 0.5% glucose have less catalase activity, i.e., capacity to break down H_2O_2 to H_2O and O_2. Note that the *cpd cya*

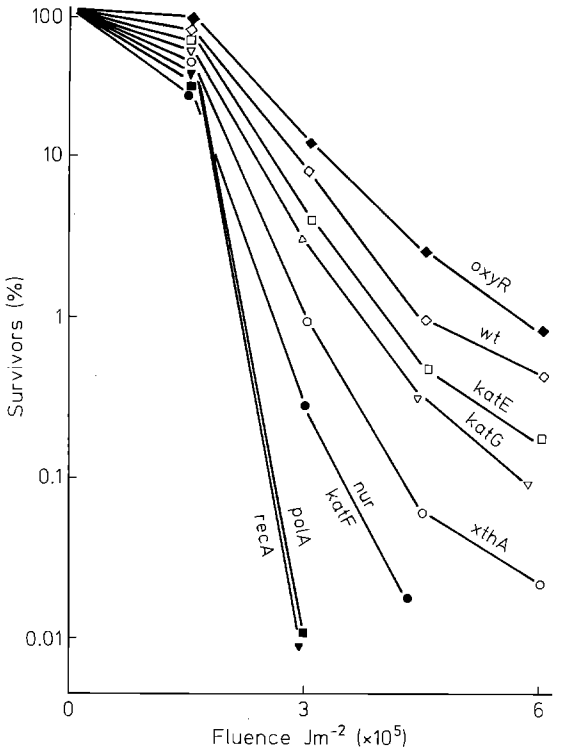

Fig. 1. Inactivation of various mutants by NUV. The procedure was the same as previously described (Hartman and Eisenstark 1978) except that a cellulose acetate filter was used to reduce radiation below 290 nm. The dose rate was 3×10^4 Joules/m^2 per min. ♦, *oxyR*; ◇, wild-type; □, *katE*; ○, *xthA*; ●, *nur* and *katF*; ■, *polA* 546; ▼, *recA*; ▽, *katG*

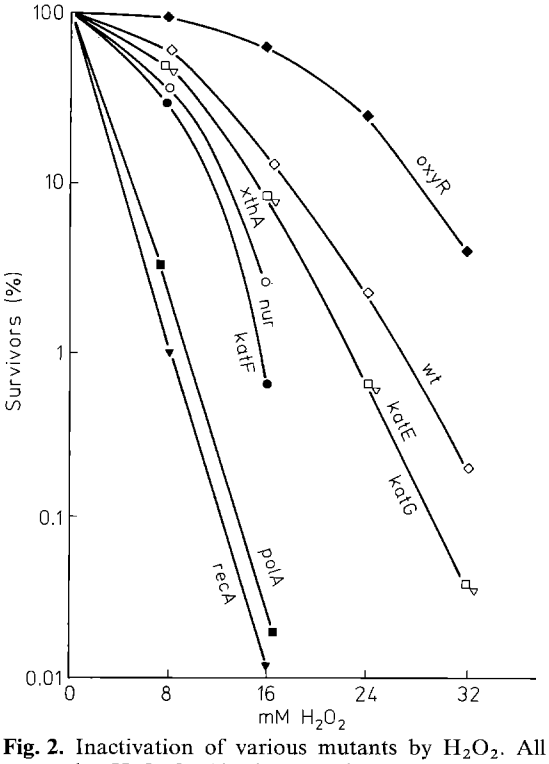

Fig. 2. Inactivation of various mutants by H_2O_2. All cells were exposed to H_2O_2 for 10 min at varying H_2O_2 concentations. Experiments were also performed with all mutants by exposing cells to 10 mM H_2O_2 for varying lengths of time (0–60 min; data not shown). The results were qualitatively the same, but exposure to varying concentrations demonstrated more distinct differences between strains. See Fig. 1 for symbols used

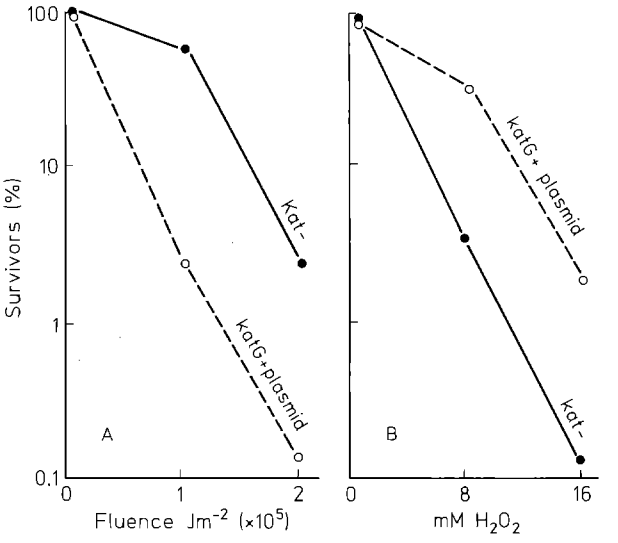

Fig. 3A, B. Comparison of *kat* (UM288) with an isogenic strain that harbors *katG*$^+$ plasmid. Inactivation by; **A** NUV; **B** H_2O_2

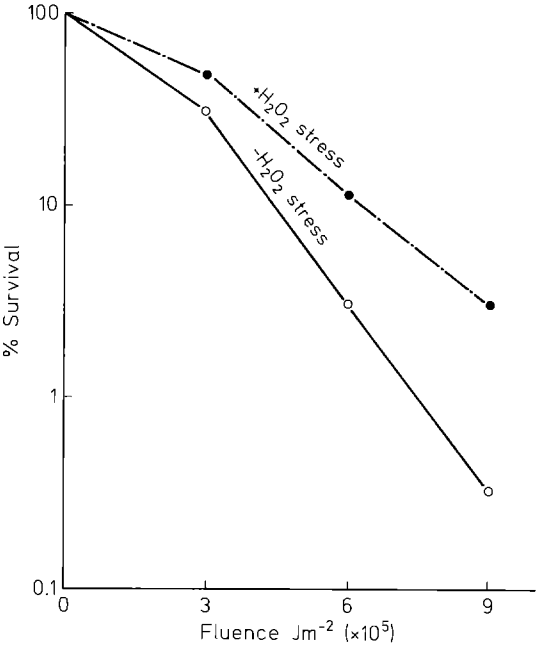

Fig. 4. Comparison of H_2O_2-stressed and non-stressed wild-type *E. coli* cells in their sensitivity to NUV. Cells were given 30 μM H_2O_2 (induction dose), and measurements were made 45 min later. *Unbroken line*, no H_2O_2 induction; *broken line*, after H_2O_2 induction

S. typhimurium mutant still shows the glucose effect, supporting the results of Richter and Loewen (1982), indicating that this does not represent classical catabolite repression involving cAMP, contrary to a previous report (Hassan and Fridovich 1978). We tested several additional cAMP mutants, none of which showed effects of catabolite repression (data not shown). Perhaps the most striking aspect of this repression is that, although cells grown in glucose are less able to breakdown H_2O_2, they are more resistant to NUV, the reverse of anticipated results. This verifies that UV-death may be due to factors in addition to endogenous H_2O_2. This is substantiated by the fact that *katE*

Table 2. Protection against NUV by pre-treatment of cells with low dose of NUV (5×10^4 Jm^{-2})

Strain	% survival after pretreatment	% survival after 6×10^5 Jm^{-2}	
		No pretreatment	Pretreatment
S. typhimurium LT2	89	1.2	0.9
S. typhimurium pep	92	1.4	12.0

and *katG* are not particularly sensitive to NUV (Table 1), although they have little catalase activity.

Of special note is the protective effect of glucose on *kat* mutants. These strains do not break down H_2O_2 but show definite increased resistance to both H_2O_2 and NUV when grown in glucose. This is evidence that the glucose protective effect is independent of the amount of endogenous catalase.

Induction by H_2O_2 of resistance to H_2O_2 and NUV

After an induction dose of 30 μM H_2O_2, cells became more resistant after 30–60 min to a challenge dose of 10 mM H_2O_2 and also had some increase in catalase. However, the catalase increase was relatively small (data not shown), consistent with the results of Demple and Halbrook (1983). The difference between a relatively small increase in catalase (about twofold) as compared with a substantial increase in NUV survival may be because induction includes many proteins in addition to catalase (Christman et al. 1985). It should be pointed out that the *katG* polypeptide is not inducible.

Cells undergoing the same pre-treatment with 30 μM H_2O_2 also become resistant to NUV (Fig. 4), an observation also made by Sammartano and Tuveson (1985) and by Tyrrell (1985). Obviously this oxidative stress evoked protective mechanisms against the toxic effects of both NUV and H_2O_2.

Induction of protective proteins by NUV

Although induction of protective proteins by NUV has been reported (Peters and Jagger 1981; Tyrrell 1985), we have been able to show protection against NUV or H_2O_2 by pre-stressing cells with NUV in only a few specific mutants, the best example of which is *S. typhimurium pep* (Strauch et al. 1985; Table 2). This differs from H_2O_2 induction which does not show this strain specificity. These results of strain specificity are contrary to expectations, since we would expect that the H_2O_2 generated by NUV in any strain would result in induction of proteins in response to stress.

Inactivation by far-ultraviolet radiation (FUV)

All of the strains listed in Table 1, grown with and without glucose, were tested for sensitivity to FUV. Glucose failed to protect cells against FUV (data not shown).

Discussion

Because the results from these experiments are different from those anticipated if catalase were a major defense enzyme for overcoming NUV toxicity, we conclude that protection against NUV is complex and that catalase probably plays only a minor role. Also, these experiments suggest that the toxic effects of NUV may differ from those of H_2O_2, and catalase may be more important in protection against H_2O_2 toxicity than against NUV toxicity.

Perhaps the most puzzling observation is that the strain containing the *katG* plasmid (and thus producing excess catalase, Table 1) is more sensitive to NUV than its non-plasmid allele (UM228), which synthesizes very little catalase (Fig. 3). A possible explanation is that, while the polypeptide produced by *katG* may have a role in quenching the cell of toxic H_2O_2, excess of this polypeptide may also act as a photosensitizer (the absorption spectrum of catalase is in the near-ultraviolet range, with a peak near 400 nm). This view is supported by the observations with the *oxyR* strain, which also synthesizes excess catalase, but with no increased resistance to NUV (Table 1). When exogenous bovine catalase is added to cells while they are being irradiated, there is no sensitization (data not shown). This may merely reflect that the catalase must be endogenous to act as a photosensitizer. It is also possible that the presence of a *recA* mutation in UM288 strain that was used might have influenced the results.

It should be noted that the slopes in Fig. 3 are about the same, but that the shoulders are different. One possible explanation is that, in NUV experiments, H_2O_2 accumulates over time, whereas cells are exposed immediately in H_2O_2 experiments.

The observation that some strains are sensitive to NUV but resistant to H_2O_2 may demonstrate that there are two defenses of the bacterial cell against NUV; to quench toxic oxygen molecules (e.g., via the products of *kat* and *sod* genes), and to repair DNA damage (via the products of *RecA*, *polA* and *xthA* genes). The products of the quenching genes may be needed only in special cases of stress. This has been emphasized by the observation that *sodAsodB* double mutants that are defective in synthesis of superoxide dismutase, grow in rich (but not minimal) medium (Carlioz and Touati 1986). The *sodAB* double mutant is sensitive to paraquat (a superoxide ion generator) and to H_2O_2. Similarly, catalase may also be needed only under particular environmental stresses, but repair of DNA damage is a major defense against NUV.

The previous observations that there is a synergistic effect between NUV and H_2O_2 might be useful in understanding the mechanisms involved in damage and recovery (Hartman 1986; Hartman and Eisenstark 1978). NUV might sensitize the cell to H_2O_2 in such a way as to block some of the recovery enzymes normally associated with H_2O_2 induction (Eisenstark 1985).

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