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recA and Catalase in H_2O_2-Mediated Toxicity in *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae **cells defective in the biosynthesis of the *recA* gene product are no more sensitive to hydrogen peroxide than wild-type cells. Although gonococci possess nearly 100-fold-greater catalase levels than *Escherichia coli*, they are more susceptible to hydrogen peroxide than this organism. The natural niche of gonococci undoubtedly results in exposure to oxidant stress; however, they do not demonstrate particularly efficient antioxidant defense systems.**

Neisseria gonorrhoeae is an important mucosal pathogen limited to the human host (4, 28). Although routinely considered a facultative anaerobic organism, several lines of evidence suggest that gonococci may thrive under strict anaerobic conditions (22). In addition, gonococcal infection is associated with a phagocytic inflammatory response likely subjecting the organisms to considerable oxidant stress (15). For these reasons, the antioxidant defenses of gonococci have attracted attention (1, 8, 29). Antioxidant defenses include scavenging enzymes (i.e., catalase, superoxide dismutase [SOD], and peroxidase) (11) and substrates, DNA repair systems (17–19, 32, 34), and adaptive competition for substrates (5). In recent work, Archibald and Duong (1) characterized the enzymatic defenses of gonococci, demonstrating that the organisms possess high levels of catalase, peroxidase, and glutathione but little or no SOD (8, 29). DNA repair systems in gonococci relevant to oxidant stress have not been specifically examined.

The antioxidant defenses of *Escherichia coli* have been extensively studied (7, 10, 17–19, 20, 30, 33, 35, 36). At least two groups have compared the relative importance of catalase and the *recA* system in the protection of *E. coli* from H_2O_2, with opposite conclusions (6, 26). The present study was undertaken to examine sensitivity to H_2O_2 in *N. gonorrhoeae* as a function of *recA* and catalase. The studies take advantage of the recent generation of a *recA* mutant of *N. gonorrhoeae* (23).

N. gonorrhoeae FA 1090 was obtained from P. F. Sparling (University of North Carolina—Chapel Hill), and the *recA* mutant of this strain was a gracious gift of M. Koomey (University of Michigan). *E. coli* K-12 RR1 and HB101 (a *recA* mutant) were provided by Janne Cannon (University of North Carolina—Chapel Hill). All gonococcal strains were maintained by daily subculture on gonococcal culture broth (GCB) agar with Kellogg's defined supplements I and II, as previously described (21). GCB was inoculated from 16- to 24-h colonies appearing on GCB agar plates and was grown to a concentration of approximately 1×10^8 to 5×10^8 viable cells per ml as determined by turbidity measurements in a Klett-Summerson colorimeter equipped with a 540-nm filter (Klett Manufacturing Inc., New York, N.Y.), a method previously employed for direct bacterial enumeration (8, 14). The *recA* mutant organisms tended to form clumps, which interfered with the enumeration of colonies required for these studies. Nonclumping variants (designated *N. gonorrhoeae recA* NC 1 through 4) were isolated by growing *recA* cells in GCB as described above, allowing the clumped cells to settle, diluting a sample of cells removed from the top of the culture, and plating them for examination of colony morphology. Nonclumping variants usually demonstrated a nonpiliated colony morphology, consistent with the observation that *recA* mutants revert to pilus expression less frequently than wild-type organisms (24). For UV irradiation experiments, cells were grown to mid-exponential phase as described above, serially diluted in GCB, and plated on GCB agar plates at various dilutions in triplicate. Plates were irradiated with 254-nm light from a General Electric GT5 germicidal lamp. Bacteria were exposed to H_2O_2 by diluting mid-log-phase cells in fresh, prewarmed (37°C) GCB (plus supplement I and 5 mM sodium bicarbonate) to 1×10^7 to 5×10^7 viable cells per ml and adding H_2O_2 (30%; Fisher Scientific, Fair Lawn, Conn.) at different concentrations for 15 min at 37°C in an atmosphere containing 5% CO_2 while shaking the cells on a rotary shaking apparatus (140 rpm). After 15 min of aerobic exposure, the cells were diluted in GCB containing 10 μg of bovine liver catalase per ml (to eliminate any remaining H_2O_2) and plated onto GCB agar. CFU were tabulated after 24 to 48 h at 37°C. For estimates of catalase and SOD activities, cells were grown to mid-log phase as described above, pelleted at 10,000 × *g* for 15 min at 4°C, and resuspended in ice-cold 50 mM potassium phosphate (KP_i), pH 7.0 or 7.8. The cells were then subjected to three lyses in an ice-cold French pressure cell (SLM Instruments, Inc., Urbana, Ill.). The extracts were clarified by centrifugation at 100,000 × *g* for 1 h, after which the cell-free supernatants were dialyzed exhaustively against 50 mM KP_i, pH 7.0 or 7.8, at 4°C. Catalase activity was measured by the method of Beers and Sizer (2). One unit of catalase activity degrades 1 μmol of H_2O_2 per min, using 8 mM H_2O_2 at room temperature. SOD activity was measured according to the method of McCord and Fridovich (27). Protein was estimated by the method of Bradford (3).

To confirm the *recA* phenotype, we first examined the "far" (254-nm)-UV sensitivity of *recA* versus wild-type gonococci. We found that the *recA* mutation rendered the cells more susceptible to UV stress than isogenic wild-type organisms (Fig. 1A). The magnitude of killing noted and the differences in susceptibility were consistent with the report of Koomey and Falkow (23). *E. coli recA* strains have been

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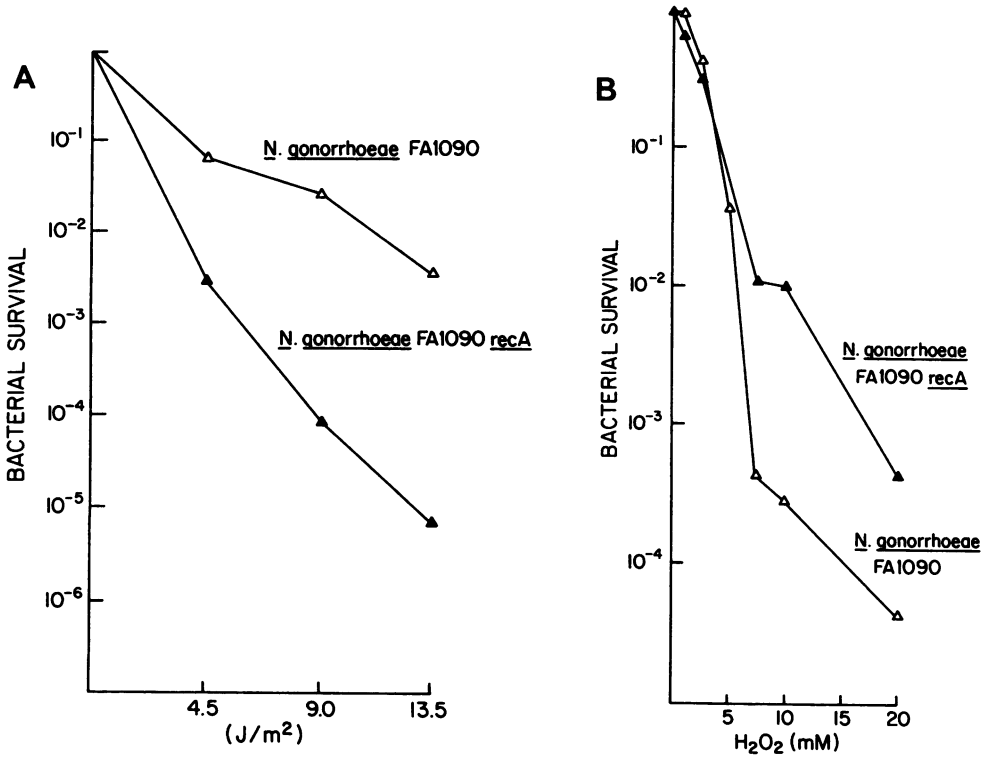

FIG. 1. (A) Survival of *N. gonorrhoeae* FA 1090 and FA 1090 *recA* after exposure to 254-nm UV light. Cells were grown to mid-log phase (1×10^8 to 5×10^8/ml), serially diluted in GCB, plated on GCB agar, and exposed to various intensities of UV light. Colonies were scored after 24 to 48 h of incubation at 37°C. Results are typical of three separate experiments, each done in triplicate. (B) Survival of aerated *N. gonorrhoeae* FA 1090 and FA 1090 *recA* after H_2O_2 challenge. Cells were grown as discussed in the text and exposed to various concentrations of H_2O_2 for 15 min at 37°C with shaking (140 rpm) in an atmosphere of 5% CO_2 prior to serial dilution in GCB (plus 10 μg of bovine liver catalase per ml) and plating on GCB agar. Colonies were scored after 24 to 48 h of incubation at 37°C. The results are typical of five separate experiments, each done in duplicate. For this work, we compared four separate nonclumping gonococcal isolates, each of which offered qualitatively and quantitatively similar results.

shown to be more sensitive to H_2O_2 than DNA repair-proficient strains (6, 17–19, 25). In particular, Imlay and Linn (17) defined killing of *E. coli* at low concentrations of H_2O_2 (1 to 2 mM) as "mode-one" killing, which they have related to deficiencies in recombinational repair and the SOS response. Mode-two killing occurs at concentrations above 25 mM and is more dependent upon H_2O_2 concentration and time of exposure. The critical sites of damage in mode-two killing are as yet unknown. We were able to duplicate the results of Imlay and Linn (17–19), using *E. coli* RR1 versus HB101 under the conditions employed in this work (data not shown). Wild-type or *recA* gonococci demonstrate greater sensitivity to H_2O_2 than *E. coli* (data not shown). However, *recA* and wild-type cells were comparably susceptible to H_2O_2, with *recA* cells demonstrating somewhat greater resistance at higher concentrations. Reliable colony counts required the use of nonclumping *recA* gonococcal variants (see above). Similar results were observed with each nonclumping variant examined ($n = 4$), making it unlikely that the results depended on this phenotype. The shape of the *recA* kill curve suggests that mode-one- and mode-two-type killing occurs in gonococci. The enhanced susceptibility of *N. gonorrhoeae* to H_2O_2 may also be due to the absence of SOD (1, 8, 29; see below). Paradoxically, mutants of *E. coli* devoid of SOD activity (*sodA sodB*) are left substantially more sensitive to mode-one killing than catalase-deficient strains or wild-type organisms (19). Theoretical explanations for the role of SOD in this setting, in addition to explanations for the mechanism of mode-one killing, have been offered (19). Mode-one killing in *E. coli* is also accentuated in mutants lacking *xthA*, *polA*, and *recB* function (17).

Whereas Carlsson and Carpenter (6) emphasized the role of *recA* in defense of *E. coli* from H_2O_2, Loewen (26) has provided data suggesting that catalase is of greater importance. In addition, resistance to moderate levels of H_2O_2 in *E. coli* strains has been attributed to catalase activity (18). We examined catalase activity in the bacteria used in studies related to *recA* activity (Table 1). Wild-type *E. coli* possessed levels of catalase and SOD activity similar to those of *recA* cells. Gonococcal catalase activity was also nearly identical in the *recA* mutant and the isogenic parent, with no detectable SOD activity in either gonococcal cell extract. Strikingly, mid-log-phase gonococci possessed nearly 100-fold more catalase activity in dialyzed cell extracts than *E. coli*. Approximately 97% of gonococcal catalase activity was localized in the cytoplasm.

In previous work with *E. coli*, catalase levels were profoundly affected by the carbon source available to the cell (13). We have been particularly interested in the effects of L-(+)-lactate on the biology of gonococcal disease (5, 12) because of the possibility that L-(+)-lactate becomes a critical substrate in inflammatory foci. The kinetics of catalase expression in gonococci grown in glucose-, lactate-, or pyruvate-containing medium were similar, but activity was greatest when lactate was the growth substrate (Fig. 2). Lactate is known to increase porphyrin- and heme-contain-

TABLE 1. Catalase and SOD activities of bacterial strains[a]

Strain	Sp act (U/mg)	
	Catalase[b]	SOD[c]
E. coli RR1	9.1 ± 0.4	17.1 ± 1.2
E. coli HB101 *recA*	8.2 ± 0.5	18.2 ± 0.2
N. gonorrhoeae FA 1090	825 ± 32	0[d]
N. gonorrhoeae FA 1090 *recA*	783 ± 43	0

[a] All organisms were grown aerobically to mid-log phase (Klett units, 80) in GCB plus 2% supplement I and 5 mM sodium bicarbonate. Cells were washed in Hanks balanced salt solution (pH 7.4), resuspended in 0.05 M KP_i (pH 7.0), and lysed by repeated passage through a French pressure cell at 4°C. The cell extracts were dialyzed exhaustively against 0.05 M KP_i (pH 7.0 or 7.8) prior to protein estimation and determination of catalase and SOD activities.

[b] Catalase activity was assayed on the basis of the decomposition of H_2O_2 monitored at 240 nm as described previously (2). One unit of catalase activity was defined as the amount which decomposes 1 μmol of H_2O_2 per min at room temperature, using 8 mM H_2O_2.

[c] SOD was assayed as described by McCord and Fridovich (27). One unit of SOD activity was defined as the amount which caused a 50% inhibition in the xanthine oxidase-catalyzed reduction of cytochrome *c*.

[d] SOD activity was not detectable in cell extracts. However, when liter quantities of cell extract were concentrated, a single, iron-cofactored SOD could be visualized on nondenaturing polyacrylamide gels (data not shown).

ing proteins in *E. coli*, *Achromobacter metalcaligenes* (9), and yeast (31). Oxidative metabolism in *E. coli* is coupled with a rise in the pH of the growth medium (13). Gonococcal growth on lactate and pyruvate caused an increase in medium pH, whereas with glucose a sharp decrease in pH was observed (Fig. 2, inset).

These studies demonstrate a remarkable difference between wild-type and *recA E. coli* and *N. gonorrhoeae* in response to exogenous H_2O_2. The *recA* mutation does not render gonococci more susceptible to H_2O_2 than wild-type bacteria. Gonococci express high levels of catalase, but generation of this enzyme does not correlate well with resistance to H_2O_2. It has been suggested that compartmentalization of catalase can effect resistance to H_2O_2 (16). The compartmentalization of this enzyme in gonococci does not mediate particular resistance to exogenous oxidant stress. The ability of gonococci to survive in inflammatory foci supports the hypothesis that the organisms are not confronted with high concentrations of H_2O_2 in vivo (5, 15).

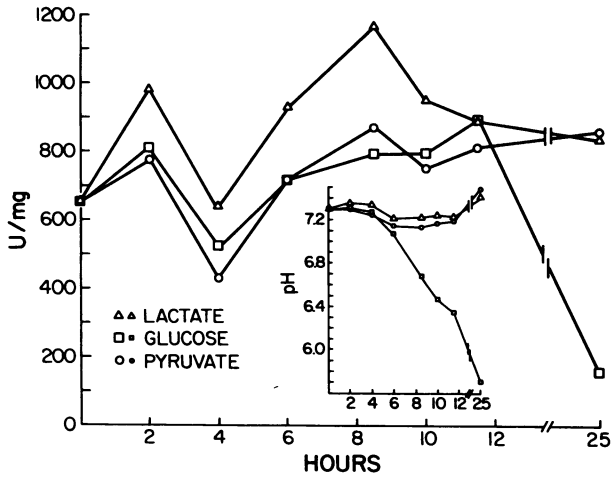

FIG. 2. Catalase activity of *N. gonorrhoeae* FA 1090 versus carbon source. Gonococci (500 ml) were grown in 2-liter Erlenmeyer flasks at 37°C in an atmosphere of 5% CO_2. An overnight culture was used to inoculate flasks to an initial reading of 5 Klett units. At intervals, cells were removed and density, medium pH, and catalase activity were measured. Cell extracts were dialyzed against 50 mM potassium phosphate (pH 7.0) and assayed for catalase activity. The results are typical of three separate experiments.

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