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Characterization of a Catalase-Deficient Strain of *Neisseria gonorrhoeae*: Evidence for the Significance of Catalase in the Biology of *N. gonorrhoeae*

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We obtained a catalase-deficient (Kat$^-$) strain of *Neisseria gonorrhoeae* isolated from a patient who had been unsuccessfully treated with penicillin. Quantitative enzyme assays and electrophoresis of cell extracts on native polyacrylamide gels subsequently stained for catalase and peroxidase activities failed to detect both enzymes. The strain exhibited no growth anomalies or unusual requirements when grown under ordinary laboratory conditions. However, the Kat$^-$ strain proved extremely sensitive to exogenous hydrogen peroxide, and analysis of the bacterial DNA after such exposure showed extensive single-strand breakage in both chromosomal and plasmid DNAs. Partial characterization of the gonococcal catalase from a Kat$^+$ laboratory strain revealed that the enzyme had the physical and chemical properties of both catalase and peroxidase.

Bacterial catalase is responsible for the decomposition and detoxification of the hydrogen peroxide (H_2O_2) that commonly results from the use of oxygen as a terminal electron acceptor (11). Hydrogen peroxide at low concentrations damages primarily DNA, causing single-strand breaks and chemical alteration of thymidine residues (1, 8, 21). Such damage, if unrepaired, may result in cell death or mutations (7, 15, 16, 20, 21, 32). Thus, catalase plays a role in maintaining the integrity and fidelity of the bacterial genome. In both *Escherichia coli* and *Salmonella typhimurium*, catalase is part of a global system regulated by *oxyR* and is involved in eliminating reduced-oxygen compounds or in limiting the damage they cause (6). The absence of *oxyR* or of *oxyR*-regulated enzymes involved in the inactivation of reduced-oxygen compounds results in a dramatic increase in the sensitivity of both of these organisms to killing or to mutation by active forms of oxygen (6, 21, 22, 33). The importance of catalase has been demonstrated directly by mutant strains of *S. typhimurium* and *E. coli* that lack catalase and peroxidase (22, 23, 35, 37) and indirectly by strains that possess suppressor mutations and that compensate for deletion of *oxyR* by overproducing catalase (13). Such second-site suppressor mutations caused reduced sensitivity to active oxygen (H_2O_2) and reduced frequencies of spontaneous mutation (13).

Since one of the major features of the early defense system of the human host is the production of hydrogen peroxide by phagocytic cells such as polymorphonuclear leukocytes (18, 34), catalase may be important for pathogenic bacteria during their initial colonization of the host and their subsequent survival. The current evidence establishing a role for catalase in the pathogenesis of a number of organisms is inconsistent. Mutants of *S. typhimurium* that contained Tn*10* insertions in *oxyR* lacked the ability to induce the enzymes responsible for inactivating reduced oxygen, were unable to survive in macrophages, and were therefore avirulent (9). Experiments to specifically define the role of catalase were performed with strains of *Staphylococcus aureus* that produced various levels of catalase activity and suggested that catalase was a significant factor in the pathogenic potential of that organism (24). However, mutants of *Shigella dysenteriae* or *Listeria monocytogenes* that lacked catalase activity (Kat$^-$) were unaffected in their abilities to infect model hosts (10, 12).

Neisseria gonorrhoeae, the organism responsible for gonococcal urethritis, produces constitutive levels of catalase activity that are high compared with those found in other organisms, including nonpathogenic and opportunistic *Neisseria* spp. (2). As for other pathogens, the advantage conferred on *N. gonorrhoeae* by catalase is uncertain, and the extent to which catalase protects that organism against hydrogen peroxide has not been demonstrated (2, 14, 17). Recently, we isolated from a clinical source and partially characterized a strain of *N. gonorrhoeae* that lacked catalase activity. With this mutant, we demonstrated that catalase significantly increased the ability of *N. gonorrhoeae* to resist in vitro killing mediated by exposure to hydrogen peroxide. Such resistance may also be of considerable importance in the survival of the organism within the human host.

MATERIALS AND METHODS

Bacterial strains. The culture of 85-015, the catalase-negative strain of *N. gonorrhoeae* used for this work, was derived from a single colony transfer from the initial Kat$^-$ culture received at the Centers for Disease Control as a positive test-of-cure culture from a male patient. Catalase-positive colonies of *N. gonorrhoeae* were later selected from the initial culture of 85-015 on the basis of their resistance to H_2O_2. The Kat$^+$ strains F62 (Pro$^-$/IB7) and 28Bl (Pro$^-$/IA8) were also used for comparison with Kat$^-$ 85-015. Strains 2820 and F622, which are Kat$^-$ derivatives of 28Bl and F62, respectively, were constructed by transformation. Strains 282 (Str^r Rif^r), 2813 (Kan^r Spc^r), and 2821 (Nal^r), which were used in transformation experiments to map *kat* in strain 2820, were derivatives of 28Bl. When necessary, all strains and isolates were confirmed as *N. gonorrhoeae* on the basis of their appearance on Gram stain, morphology, oxidase reaction, and carbohydrate utilization patterns and by a

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commercial DNA probe (Genprobe, San Diego, Calif.). Auxotypes and protein I serovars were determined as described elsewhere (20, 31). All strains were cultivated on GC base agar (GCBA) supplemented with 1% IsoVitaleX (Baltimore Biological Laboratories, Baltimore, Md.) and 1% fetal bovine serum at 37°C in 5% CO_2.

Determination of enzymatic activities. The amounts of catalase activity present in extracts of sonically disrupted *N. gonorrhoeae* were determined by the method of Beers and Sizer (4). The catalase and peroxidase activities present in gonococcal extracts were also determined by electrophoresis on nondenaturing polyacrylamide gels by staining for both of these enzymatic activities as described elsewhere (27, 36). The absence of superoxide dismutase in both the Kat^+ and Kat^- strains was similarly confirmed (3, 27, 29).

Partial characterization of gonococcal catalase. The catalase activity present in crude extracts of 28Bl was partially characterized as follows. The pH optimum for catalase activity was determined by diluting the crude extract 100-fold into 0.05 M phosphate buffer adjusted to the desired pH within a pH range of 3 to 12. The activity of the enzyme was determined as described above at the same pH as the enzyme dilution. The sensitivity of gonococcal catalase to the catalase inhibitor 3-amino-1,2,4-triazole was determined by preincubating the enzyme with 4 mM cysteine and 20 mM inhibitor. The amount of catalase converted to the inactive catalase-hydrogen peroxide II form (26) was determined by assaying the remaining activity in the extract at 15-min intervals. The sensitivity of gonococcal catalase to inactivation by organic solvents was also determined. The crude extract was thoroughly mixed with ethanol-chloroform (5:3) in equal volumes. The precipitated protein and organic solvents were removed by centrifugation. The remaining soluble proteins were then washed three times with equal volumes of buffer in a Centriprep 10 concentrator (Amicon, Beverly, Mass.) to eliminate residual organic solvents. The catalase activities remaining in the washed extracts were then determined.

Survival of *N. gonorrhoeae* in the presence of hydrogen peroxide. The ability of Kat^+ and Kat^- strains to survive exposure to H_2O_2 was tested in a fashion similar to that described elsewhere (15, 16). A suspension containing 5×10^8 CFU/ml from an overnight culture was made in Trypticase soy broth (TSB). The surfaces of each of three supplemented GCBA plates were inoculated with 0.5 ml of the suspension. The inoculated plates were incubated for 4 h. The bacteria were then harvested with a swab and suspended in TSB to a density of 2×10^8 to 4×10^8 CFU/ml. Samples (200 μl each) were transferred to 1.5-ml microcentrifuge tubes. A 3% stock solution of H_2O_2 was diluted in TSB to twice the desired final concentration immediately prior to use. A 200-μl volume of each diluted H_2O_2 solution was then added to a separate microcentrifuge tube that contained 200 μl of the cell suspension. The tubes were incubated at room temperature for 10 min. The treatment was terminated by transferring 100 μl of each H_2O_2-treated suspension to 0.9 ml of gonococcal genetic medium (31) that contained 1,400 U of bovine catalase (Sigma, St. Louis, Mo.). Subsequent dilutions to enumerate surviving bacteria were performed in TSB, and the samples were plated on supplemented GCBA. Colonies were counted after 36 h of incubation.

Determination of H_2O_2-induced damage to bacterial DNA. The extent of breakage of gonococcal DNA resulting from exposure to H_2O_2 was analyzed by alkaline agarose gel electrophoresis. The bacteria were grown and exposed to H_2O_2 as described above except that 1,400 U of catalase was added directly to each reaction and no subsequent dilutions were performed. The cells were recovered by centrifugation and then suspended in 25 μl of H_2O_2. The bacteria were lysed, and their DNAs were denatured by the addition of 25 μl of an alkaline lysis solution consisting of 0.1 N NaOH, 0.002 M EDTA, 5% (wt/vol) Ficoll, and 0.08% (wt/vol) bromcresol green. The denatured DNA was analyzed by electrophoresis of 14 μl of each sample on 0.75% alkaline agarose gel as described by Maniatis et al. (25). After electrophoresis, the gels were agitated in 350 ml of a solution of 0.05 M Tris with 0.03 M NaCl (pH 6.0) to neutralize it, and then the DNA was stained with ethidium bromide.

Introduction of the Kat^- mutation into other strains of *N. gonorrhoeae*. The Kat^- mutation was transformed from 85-015 into both F62 and 28Bl by congression, which depends on the uptake of multiple fragments of donor DNA by a single recipient cell. A Rif^r mutant of 85-015 was selected on supplemented GCBA that contained 50 μg of rifampin per ml. After the mutant was subcultured for purification and the presence of the Kat^- mutation was confirmed in the resistant transformant, DNA was purified from it and sheared to an average length of 10 kb. Saturating amounts (12 μg/5×10^8 CFU) of sheared DNA from 85-015 Rif^r were used to transform piliated cells of 28Bl and F62 to Rif^r. The selective plates that contained the Rif^r transformants were flooded with 3% H_2O_2 to determine which of them had simultaneously acquired the Kat^- mutation from the donor. Rif^r Colonies that failed to generate gas were quickly picked and streaked onto supplemented GCBA. Individual colonies from each of the streaked plates were picked onto duplicate master plates. After incubation, one set of plates was used to test for catalase production among the recombinants, and the second set served to provide stock cultures.

RESULTS

Strain 85-015 exhibited neither catalase activity nor superoxol activity (36) when heavy growth of the organism on supplemented GCBA was flooded with either 3 or 30% H_2O_2. In addition, no catalase activity was detected when cell extracts of 85-015 were assayed by the method of Beers and Sizer (4). In contrast, extracts of strain 28Bl contained 800 to 950 U of catalase activity per mg of cell protein when assayed by the same method. Similarly, native polyacrylamide gel electropherograms of extracts of 28Bl and 85-015 stained for catalase activity confirmed the absence of detectable levels of enzyme in strain 85-015 (Fig. 1). Peroxidase activity, which was previously described in *N. gonorrhoeae* (2), was also not detected in the extracts of the Kat^- strain but was present in extracts of 28Bl; the band that contained the peroxidase activity migrated with the same relative mobility as the band that contained the catalase activity (Fig. 1). Neither strain possessed detectable levels of superoxide dismutase (data not shown). Growth of strain 85-015 on supplemented chocolate agar or supplemented GCBA that contained 5 μg of hemin per ml did not reverse the Kat^- phenotype. Because strain 85-015 lacked detectable amounts of catalase and peroxidase and because both enzymatic activities demonstrated an equal degree of electrophoretic mobility on native polyacrylamide gels, we considered that both activities might be properties of the same enzyme. Gonococcal catalase exhibited the type of resistance to 3-amino-1,2,4,-triazole (Fig. 2) previously observed for bacterial hydroperoxidases (2, 25). However, the catalase in extracts of *N. gonorrhoeae* exhibited constant activity from

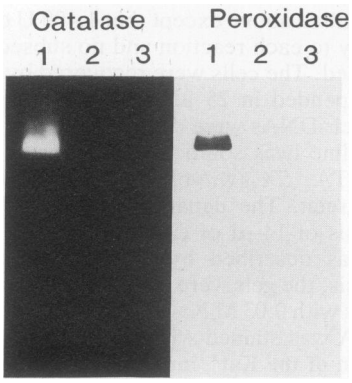


FIG. 1. Analysis of catalase and peroxidase activities in sonicates of *N. gonorrhoeae*. Duplicate samples of extracts of 28Bl (Kat$^+$) (lane 1), 85-015 (Kat$^-$) (lane 2), and 2820 (28Bl Kat$^-$) (lane 3) were subjected to electorphoresis on native polyacrylamide gel. The gel was divided, and the left three lanes were stained for catalase activity, while the right three lanes were stained for peroxidase activity with diaminobenzinine and H_2O_2.

pH 4 to pH 12 (data not shown), with sharp declines below pH 3 and above pH 12. The gonococcal catalase also showed itself to be highly resistant to organic solvents. In fact, the specific activity of catalase present in gonococcal extracts after exposure to ethanol-chloroform was higher than before exposure.

The absence of catalase activity did not appear to detectably affect the growth or viability of strain 85-015. Colonies produced by strain 85-015 were similar in size to those produced by strains 28Bl and F62 after 18 to 20 h of incubation. When suspensions of each of the three strains were adjusted to the same optical density, diluted, and spread onto supplemented GCBA, the viable count obtained for each of the strains was the same: 2×10^8 to 4×10^8 CFU/ml at an optical density at 530 nm of 0.3. The frequencies of spontaneous mutation of 85-015 to resistance to rifampin (50 μg/ml) and streptomycin (100 μg/ml) were determined by spreading 0.1-ml samples of a suspension of

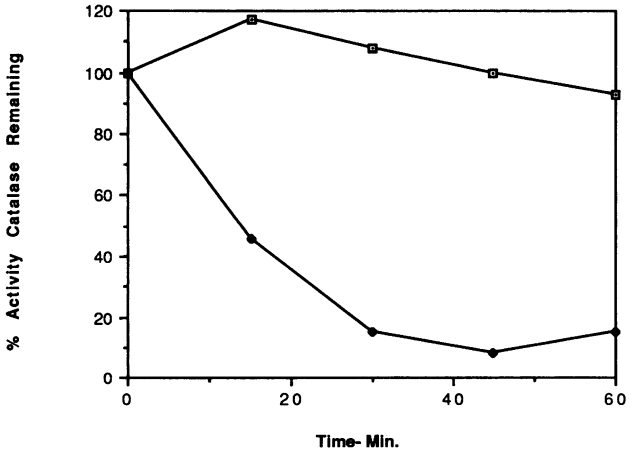


FIG. 2. Insensitivity of gonococcal catalase to inactivation by 3-amino-1,2,4-triazole. Gonococcal extracts containing catalase activity (⊡) were exposed to cysteine with 3-amino-1,2,4-triazole and then assayed for activity. Purified bovine catalase (◆) was used as the control for inactivation.

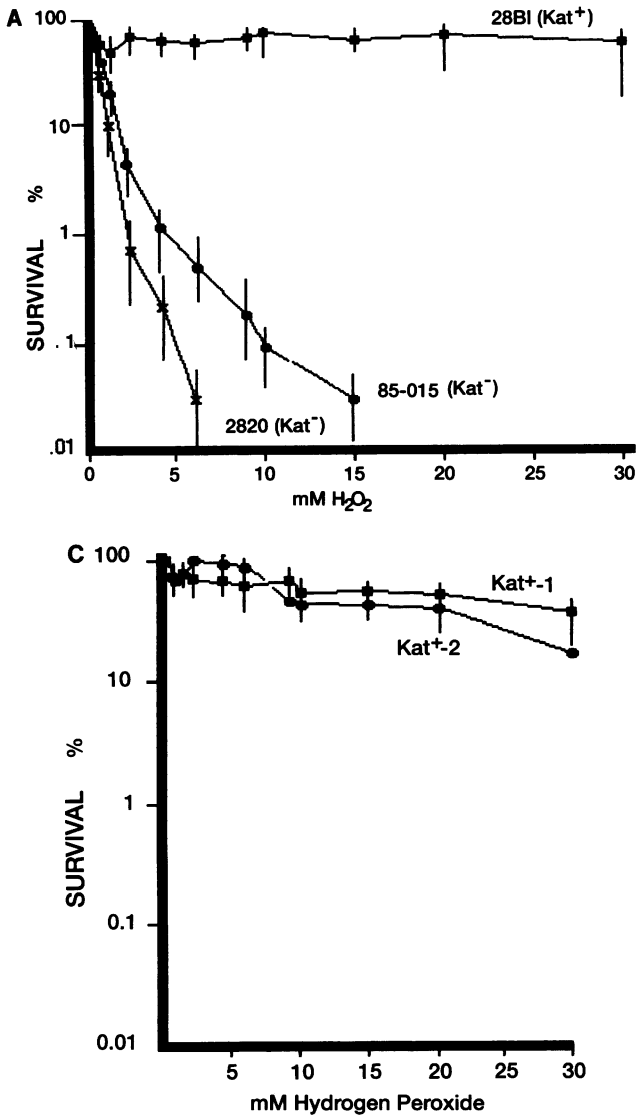
log-phase cells (approximately 2×10^{10} CFU/ml per sample) onto supplemented GCBA that contained the appropriate antibiotic. The numbers of viable cells in the suspensions were also determined. The frequencies obtained for both mutations in strain 85-015 (2.0×10^{-9} Rif^r colonies and 2.2×10^{-9} Str^r colonies per CFU) were essentially identical to the frequencies obtained for strain 28Bl (1.7×10^{-9} Rif^r colonies and 1.1×10^{-9} Str^r colonies per CFU).

Using two different approaches, we attempted to determine whether the Kat mutation in 85-015 reverted spontaneously or could be suppressed. Initially, we inoculated 30 GCBA plates containing supplements with a lawn of about 5.0×10^4 CFU from a culture derived from a single colony of 85-015. The inoculated plates were incubated for 18 h to allow microcolonies to develop and then flooded with 3% H_2O_2 to detect Kat$^+$ colonies. No Kat$^+$ colonies were detected among the approximately 1.5×10^6 colonies screened. In an alternative approach, 4×10^7 CFU of the same subculture of 85-015 as described above was exposed to 6 mM H_2O_2 for 10 min and then spread onto supplemented GCBA. After incubation, any resulting colonies were tested individually for the presence of catalase. No Kat$^+$ colonies were detected among 500 colonies tested. However, when the initial culture of strain 85-015 rather than a single colony subculture was subjected to the same treatment, 6 Kat$^+$ colonies were recovered from 320 colonies tested. The auxotypes and serovars of three of these colonies were determined to be the same as those of strain 85-015 (Pro$^-$/IB3).

The sensitivities of strain 85-015 and the Kat$^+$ strains to H_2O_2 were determined as described in Materials and Methods. The data presented in Fig. 3A show that 85-015 was killed even at low concentrations of H_2O_2 and suffered considerable loss of viability at concentrations of >4 mM. On the other hand, strains F62 and 28Bl were relatively unaffected by 30 mM H_2O_2 (Fig. 3A and B). Doubling the exposure time to 20 min did not significantly increase the amount of killing (20%) (data not shown). As expected, the Kat$^+$ isolates obtained from the original culture received (85-015K1 and 85-015K2) were not as sensitive to H_2O_2 as strain 85-015 (Fig. 3C). Interestingly, all of the Kat$^+$ strains tested appeared to exhibit a slightly increased but reproducible (75% of repeated assays) killing at very low concentrations (0.5 to 1.0 mM) of H_2O_2.

To determine the degree to which gonococcal catalase protected the bacterial DNA from H_2O_2-induced single-strand breakage, suspensions of strains 85-015, 28Bl, F62, 85-015K1, and 85-015K2 were exposed to H_2O_2 and then subjected to alkaline lysis. The DNA contained in the lysates was analyzed for the extent of single-strand breakage by alkaline agarose gel electrophoresis. The DNA of 85-015 was damaged by low concentrations of H_2O_2 (Fig. 4B). Single-strand breaks in the bacterial chromosome occurred at 1 mM H_2O_2, and approximately 50% of the 4.2-kb cryptic plasmid DNA was converted to the open circular form by exposure of the bacteria to 2 mM H_2O_2. In contrast, there was no evidence for significant nicking or fragmentation of chromosomal DNA or cryptic plasmid DNA of strain 28Bl at less than 20 mM H_2O_2 (Fig. 4A).

Genetic analysis of the Kat$^-$ mutation in strain 85-015 was prevented by the inability of the strain to produced piliated competent colonies. It was also not possible to detect the transfer of chromosomal antibiotic resistance markers from a donor strain of *N. gonorrhoeae* into 85-015 either by conjugation mediated by the gonococcal conjugative plasmid (30) or by electroporation (19). Therefore, we sought to



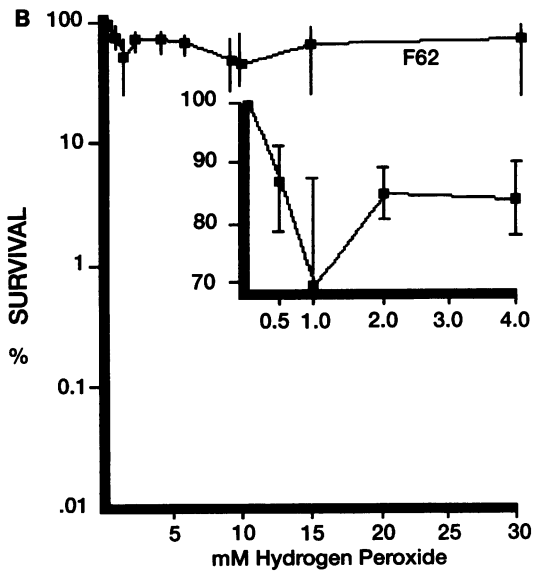


FIG. 3. Sensitivities of Kat$^+$ and Kat$^-$ strains of *N. gonorrhoeae* to H_2O_2. Suspensions of gonococci were exposed to increasing concentrations of H_2O_2 for 10 min, and the numbers of viable bacteria were determined as described in the text. (A) Kat$^-$ strain 85-015, Kat$^+$ strain 28Bl, and Kat$^-$ strain 2820, a derivative of 28Bl; (B) Kat$^+$ strain F62; (C) two Kat$^+$ isolates selected from the initial culture of 85-015. Each point plotted represents the average of five separate experiments. The error bars in Fig. 3C are for 86-015K2 only.

introduce the Kat$^-$ mutation from 85-015 Rif^r into both F62 and 28Bl by congression as described in Materials and Methods. We were able to obtain four Kat$^-$ Rif^r transformants of 28Bl and three similar transformants of F62 from among approximately 4,000 Rif^r transformants screened for each of the two recipients. Two isolates, designated 2820 and F622, were chosen for characterization and found to be devoid of detectable catalase and peroxidase activities (Fig. 1). Strain 2820 appeared more sensitive to H_2O_2 than the original mutant strain 85-015 (Fig. 3A), and the extent of damage to the DNA of 2820 by H_2O_2 was indistinguishable from that observed for 85-015.

Unlike 85-015, strains 2820 and F622 produced piliated (P$^+$) colony types and exhibited the same frequencies of transition between P$^+$ and P$^-$ as the Kat$^+$ parent strains. Thus, it was possible to attempt transformation of Kat$^-$ strains to Kat$^+$. Potential Kat$^+$ transformants were plated onto supplemented GCBA and incubated for 20 h. Kat$^+$ colonies were detected by flooding the plates that contained potential transformants with 3% H_2O_2. Such transformants were obtained at a frequency of 0.2 transformants per 100 CFU when strain 28Bl was used as the DNA donor. As expected, when 85-015 was used as the donor, no Kat$^+$ recombinants were detected. Some linkage data were obtained by using strains 282 (Str^r), 2812 (Spc^r), and 2821 (Nal^r) donors for the three antibiotic resistances to 2820. When 2812 was the donor, 3.8% of the selected Spc^r recombinants were also Kat$^+$ (4 Spc^r Kat$^+$ colonies per 104 Spc^r colonies tested). When strain 282 (Str^r) or 2821 (Nal^r) served as donor, neither antibiotic resistance marker showed detectable linkage to *kat* (0 Kat$^+$ colonies per 104 colonies tested).

DISCUSSION

A strain of *N. gonorrhoeae* isolated from a male patient with gonococcal urethritis lacked detectable catalase activity in both qualitative and quantitative assays. The fact that catalase activity is ubiquitous among gonococci suggests that the strain, designated 85-015, contained a mutation that resulted in the loss or severe reduction of catalase activity. The absence of detectable catalase activity in strain 85-015, the absence of observable pleiotropic effects such as the loss of cytochrome oxidase activity, and the apparent inability of hemin to reverse the Kat$^-$ phenotype indicated that the defect likely involved mutation of a regulatory sequence (or gene) or mutation of the structural gene for gonococcal catalase rather than loss of the ability to synthesize the prosthetic group. Studies that demonstrated high constitutive levels of catalase in *N. gonorrhoeae* (2, 14, 39) and a relatively limited capacity to repress or elevate levels of the enzyme in response to oxidative stress (39) suggest that the catalase gene in *N. gonorrhoeae* is not subject to tight regulation. Evidence for such loose regulation of catalase does not prove but does appear to favor the explanation that the mutation present in 85-015 involves either the structural gene for catalase or an immediate promoter region rather

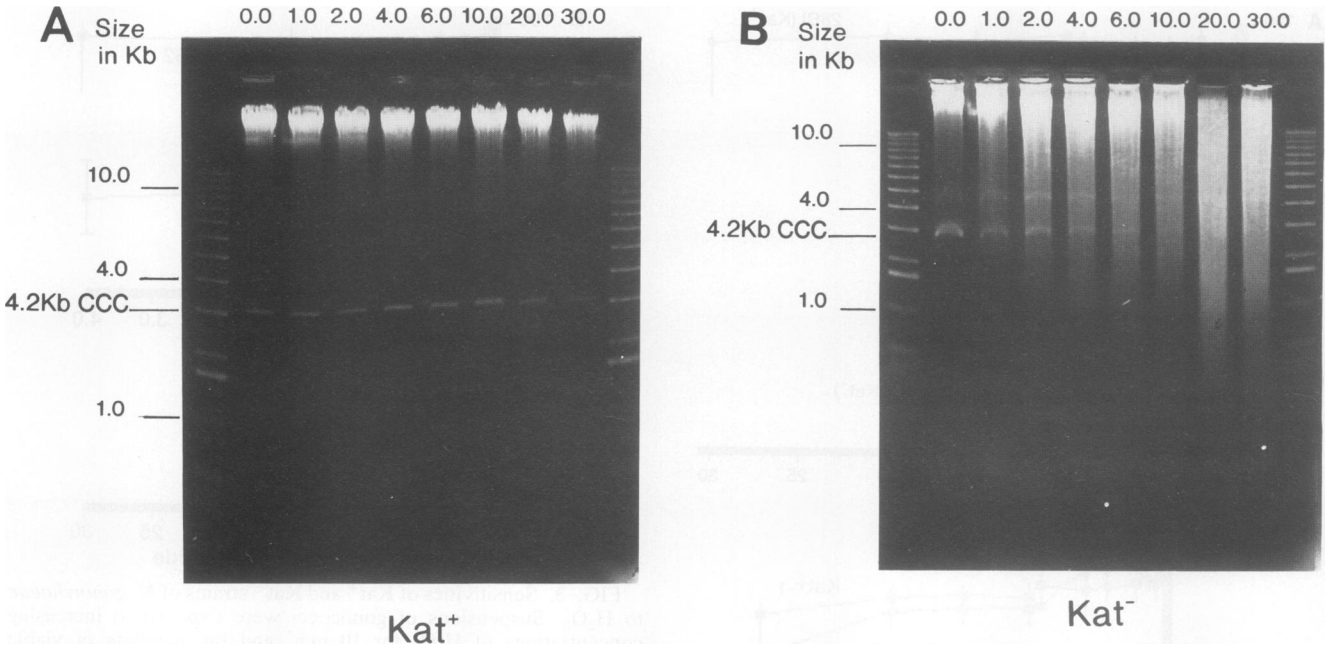


FIG. 4. Damage of DNA in Kat$^+$ and Kat$^-$ strains of *N. gonorrhoeae* by exposure to H_2O_2. Suspensions of gonococci were exposed to H_2O_2, cells were subsequently lysed under alkaline conditions, and the DNA was analyzed for single-strand breaks by alkaline agarose gel electrophoresis. The size standard was denatured kilobase ladder DNA (Bethesda Research Laboratories, Bethesda, Md.). (A) Results of treatment of 28Bl; (B) results for 85-015. CCC, covalently closed circular DNA.

than a gene(s) such as an inducer or a sigma factor that functions in *trans* to regulate catalase expression.

The results of transformation experiments demonstrating that the Kat$^-$ mutation could be transferred by congression to strain 28Bl to construct the Kat$^-$ derivative 2820 and the subsequent genetic crosses that showed that 2820 could then be efficiently transformed to Kat$^+$ with weak linkage between *spc* and the Kat$^-$ mutation argue that the mutation(s) was confined to a small region of the gonococcal chromosome. It is also important that no pleiotropic effects attended the transfer of the Kat$^-$ mutation from 85-015 to 28Bl or F62.

The availability of Kat$^-$ strain 85-015 has enabled us to gain further insight into the effects of reduced-oxygen compounds on *N. gonorrhoeae*. In addition to lacking catalase activity, the Kat$^-$ strain 85-015 lacked both superoxol and peroxidase activities, which were earlier described for *N. gonorrhoeae* (2, 38). The superoxol activity would be predicted to be a property of the gonococcal catalase and thus would be lost along with catalase. However, earlier work done on the *N. gonorrhoeae* catalase attributed the peroxidase activity to a separate enzyme (2). Three lines of evidence suggest that the gonococcal catalase is actually a hydroperoxidase and possesses both activities. First, both catalase and peroxidase activities appeared to comigrate on native polyacrylamide gels, and second, both catalase and peroxidase activities were lost not only by strain 85-015 but also by the constructed strains 2820 and F622. Partial characterization of the gonococcal enzyme also suggested that both activities might belong to the same enzyme. Like other bacterial hydroperoxidases, this enzyme was unaffected by the catalase inhibitor 3-amino-1,2,4-triazole (2, 5, 26, 28). However, the gonococcal enzyme, like mammalian and bacterial catalases and unlike bacterial peroxidases, exhibited essentially constant activity over a wide pH range and was unaffected by exposure to an ethanol-chloroform mixture (5, 27).

Although *N. gonorrhoeae* 85-015 contained cytochrome oxidase activity (2) and presumably remained capable of respiration using molecular oxygen with the associated production of reduced-oxygen species (11), the absence of catalase did not appear to have any effect on its survival when the organism was grown at ambient levels of O_2. Neither the growth rate nor the viability of strain 85-015 or 2820 was adversely affected relative to those of Kat$^+$ strain 28Bl. It has been previously shown that the production of elevated levels of catalase by an OxyR$^-$ deletion mutant of *E. coli* reduced the frequencies of spontaneous mutations (13). Therefore, we postulated that the absence of catalase from strain 85-015 might result in increased frequencies of spontaneous mutations. Data for spontaneous mutations at two different loci demonstrated that this was not the case. However, we did not determine the mutation frequencies when 85-015 was grown in an oxygen-enriched atmosphere or exposed to exogenous H_2O_2.

The effect of the absence of catalase on both the viability and the integrity of the DNA of *N. gonorrhoeae* was assessed by exposing both Kat$^-$ strains (85-015 and 2820) and four Kat$^+$ strains to H_2O_2. In contrast to the Kat$^+$ strains, which exhibited no greater than 70% reduction in viability when exposed to 30 mM H_2O_2, both Kat$^-$ strains appeared sensitive to concentrations of H_2O_2 as low as 1 mM. These results suggested that catalase effectively protected *N. gonorrhoeae* against the effects of H_2O_2 over a wide range of concentrations. The fact that 2820 was no less sensitive to H_2O_2 than 85-015 strongly suggested that catalase was the sole protective mechanism involved. Interestingly, Kat$^+$ *N. gonorrhoeae* appeared to be more sensitive

to very low concentrations of H_2O_2 and to exhibit a phenomenon observed in *E. coli* called mode I killing (15, 16).

The amount of H_2O_2-mediated damage, as determined by the degree of single-strand breakage of both plasmid and chromosomal DNAs of 85-015, was far greater than the amount of damage done to the DNA of strain 28Bl and reflected the results of the experiments that monitored viability. Thus, the gonococcal genome appears to be a significant target of H_2O_2-mediated damage, and the presence of catalase protects both the integrity of the bacterial genome and viability. Other investigators have obtained different results. Hasset et al. (14) observed that FA1090 (Kat^+) exhibited far greater sensitivity to H_2O_2 than was observed for 28Bl (Kat^+). Although the conditions of H_2O_2 exposure used in both sets of experiments were similar, the bacteria were grown under different conditions, and this may account for the observed differences in results. In fact, when we determined the susceptibility of strain FA1090 to H_2O_2 under the same conditions we used for 28Bl and F62, FA1090 appeared only slightly more sensitive to H_2O_2 stress than strains 28Bl and F62. However, the exact nature of the differences in results obtained with the two protocols remains to be investigated further.

Although catalase appeared protective, the role it plays in gonococcal pathogenesis also remains uncertain. The differing responses the Kat^+ and Kat^- organisms exhibited toward exogenous H_2O_2 suggested that catalase might have a significant role either by increasing the survival of the organism in the presence of hydrogen peroxide-producing phagocytic cells (18, 34), especially early in the course of infection, or by allowing the gonococcus to colonize genital-tract tissues in the presence of such natural flora as H_2O_2-producing lactobacilli. While a significant role for catalase in the pathogenesis of *N. gonorrhoeae* might appear inconsistent with the isolation of a strain that lacked the enzyme, it is important to note that the initial culture of 85-015 was not a pure Kat^- culture but contained a significant number of cells of a Kat^+ strain that exhibited the same serovar and auxotype as strain 85-015. It may well be that strain 85-015 alone is incapable of causing an infection but that under some circumstances it can participate in a coinfection when protected by organisms that produce normal levels of catalase.

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