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Hypochlorous Acid Stress in *Escherichia coli*: Resistance, DNA Damage, and Comparison with Hydrogen Peroxide Stress

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We have investigated the mechanisms of killing of *Escherichia coli* by HOCl by identifying protective functions. HOCl challenges were performed on cultures arrested in stationary phase and in exponential phase. Resistance to HOCl in both cases was largely mediated by genes involved in resistance to hydrogen peroxide (H_2O_2). In stationary phase, a mutation in *rpoS*, which controls the expression of starvation genes including those which protect against oxidative stress, renders the cells hypersensitive to killing by HOCl. RpoS-regulated genes responsible for this sensitivity were *dps*, which encodes a DNA-binding protein, and, to a lesser extent, *katE* and *katG*, encoding catalases; all three are involved in resistance to H_2O_2. In exponential phase, induction of the *oxyR* regulon, an adaptive response to H_2O_2, protected against HOCl exposure, and the *oxyR2* constitutive mutant is more resistant than the wild-type strain. The genes involved in this *oxyR*-dependent resistance have not yet been identified, but they differ from those primarily involved in resistance to H_2O_2, including *katG*, *ahp*, and *dps*. Pretreatment with HOCl conferred resistance to H_2O_2 in an OxyR-independent manner, suggesting a specific adaptive response to HOCl. *fur* mutants, which have an intracellular iron overload, were more sensitive to HOCl, supporting the generation of hydroxyl radicals upon HOCl exposure via a Fenton-type reaction. Mutations in recombinational repair genes (*recA* or *recB*) increased sensitivity to HOCl, indicative of DNA strand breaks. Sensitivity was visible in the wild type only at concentrations above 0.6 mg/liter, but it was observed at much lower concentrations in *dps recA* mutants.

Chlorination is the most widely used bactericidal agent to disinfect drinking water and protect distribution systems. In aqueous environments, there is an equilibrium between two forms (collectively referred to as free chlorine), un-ionized hypochlorous acid (HOCl) and hypochlorite ions (ClO^-). Their ratio depends on pH and temperature. HOCl is the more reactive of these two forms (2).

Upon stimulation during an oxidative burst, neutrophils and macrophages release cell-damaging concentrations of HOCl involved in antimicrobial action (19). HOCl is formed by the myeloperoxidase-catalyzed peroxidation of chloride ions $H_2O_2 + Cl^- \rightarrow HOCl + OH^-$ at the expense of hydrogen peroxide (H_2O_2) (31).

Hypochlorous acid is generally considered to be a highly destructive, nonselective oxidant which reacts avidly with a variety of subcellular compounds and affects metabolic processes (2, 3, 41). It acts on membranes, changing their permeability (46, 54), inhibits transport (7), fragments proteins (49), and reacts with nucleotides (10, 17). It inactivates enzymes, iron-sulfur clusters appearing as privileged targets (8). It was shown that ATP production is abolished by selective oxidation of F_1-ATP synthase (7, 21). Low concentrations selectively and rapidly inhibit cell division (42). There is some evidence that HOCl can attack DNA (55). A few reports have shown that chloramine, which can be generated in vivo by HOCl (10, 49), causes DNA damage (10, 44, 45, 51), and genotoxicity of free chlorine and chloramines in drinking water was detected with amphibians (27). Recently, Candeias et al. (12) have shown that in vitro, hypochlorous acid can also directly generate hydroxyl radicals via a Fenton-type reaction (24), $HOCl + Fe(II) \rightarrow {}^{\cdot}OH + Cl^- + Fe(III)$, and that in the presence of superoxide anions, hypochlorous acid leads to production of hydroxyl radicals in a reaction, $HOCl + O_2^{\cdot -} \rightarrow {}^{\cdot}OH + Cl^- + O_2$ (11), similar to the Haber-Weiss reaction, $H_2O_2 + O_2^{\cdot -} \rightarrow {}^{\cdot}OH + HO^- + O_2$ (9). Khan and Kasha (29, 30) have shown that oxygen singlet, a highly reactive, potentially DNA damaging species, is generated in vitro via a Haber-Weiss reaction and upon acidification of an HOCl solution. Furthermore, free chlorine or chloramine can react with H_2O_2 in a chemiluminescent reaction generating singlet oxygen (22, 23). Thus, in vitro, HOCl and H_2O_2 generate many of the same reactive oxidative species (hydroxyl radicals and oxygen singlet) which can attack DNA.

The ability of bacteria to resist and adapt to the presence of H_2O_2 has been extensively studied. In exponentially growing cells, exposure to low concentrations of H_2O_2 leads to the induction of proteins important for cellular defense against the oxidative stress produced by higher concentrations (16). Several of these proteins, including catalase (36), alkyl hydroperoxide reductase (48), glutathione reductase (20), and Dps (4), a nonspecific DNA-binding protein, have been shown to be under the control of a transcriptional activator, OxyR (5, 14). Upon starvation or entry into stationary phase, protective functions against H_2O_2 are induced (28) under the control of *rpoS* as part of the many functions which enable bacteria to survive starvation (32, 34). In contrast, mechanisms of resistance and adaptation of bacteria to HOCl remained largely unknown.

In this work, we investigated the resistance of *Escherichia coli* to hypochlorous acid. We examined whether the *rpoS*- and *oxyR*-governed adaptive response to H_2O_2 protect against hypochlorous acid stress and whether exposure to low HOCl concentrations adapts cells to further H_2O_2 stress. Results presented here indicate overlapping defense systems to HOCl and H_2O_2 and support the idea that in vivo as in vitro, H_2O_2 and HOCl generate common deleterious oxidative species which can damage DNA.

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MATERIALS AND METHODS

Bacterial strains. All strains are *E. coli* K-12 derivatives. Mutations in various genes were introduced by P1 transduction in the same parental strain, MG1655 (6), using P1 *vir* and selecting for an antibiotic resistance associated with the following mutations: resistance to chloramphenicol for Φ(*sodA'-'lacZ*)*49* (13), resistance to kanamycin for *dps*::*kan* (4), Δ*fur*::*kan* (53), Δ*oxyR*::*kan* (52), Φ(*sodB-kan*)Δ*2* (13), and Δ*ahpF*::*kan* (a gift from R. Hayward), and resistance to tetracycline for *katE*::Tn*10* (35), *katG*::Tn*10* (36), *recB268*::Tn*10* (37), and *rpoS*::Tn*10* (35). The Δ*recA306* (53) strains were generated by cotransduction of the *recA* allele with *srl*::Tn*10*; selection was for tetracycline resistance, and the cotransduction was confirmed by UV sensitivity. *oxyR2* (14) was cotransduced with *zij*::Tn*10* or *purD81*::Tn*5*, and tetracycline- or kanamycin-resistant transductants were screened for H_2O_2 resistance. The *lexA*(Ind$^-$) (53) strains were constructed by cotransduction of the *lexA*(Ind$^-$) allele with *malB*::Tn*9*; selection was for chloramphenicol resistance, and transductants were screened for UV sensitivity.

Reagents and hypochlorous acid assay. All chemicals used were of analytical grade. *N,N*-Diethyl-*p*-phenylenediamine and sodium thiosulfate were purchased from Sigma Chemical Co.; sodium hypochlorite (NaClO) and hydrogen peroxide were from Aldrich Chemical Company, Milwaukee, Wis. Beef liver catalase was purchased from Boehringer Mannheim. Fresh solutions were prepared daily in distilled water and used immediately.

The concentration of free chlorine (hypochlorous acid plus hypochlorite ion) was determined both iodimetrically (15) and colorimetrically (1). NaClO solution in distilled water was stable for several hours. Free chlorine consumption by the medium and the bacteria was determined. When 1 mg of NaClO per liter was added to phosphate buffer, 0.6 mg of free chlorine per liter was detected after 20 min. In the presence of 10^6 bacteria per ml, no more free chlorine was detected after 20 min. Higher concentrations of bacteria interfered with the assay.

Growth, hypochlorous acid challenge conditions, and adaptation. Cells were grown in M63 (43) plus 0.2% glucose at 25°C in a rotary shaking water bath at 200 rpm. Erlenmeyer flasks used for HOCl treatment were washed with sulfochromic acid.

(i) Hypochlorous acid challenge of cells collected in exponential growth phase. At an optical density of 0.09 at 600 nm (5×10^7 bacteria per ml), the cells were spun down at 5,000 rpm for 10 min at 4°C, washed twice with cold 0.05 M phosphate buffer (pH 7.1), and resuspended in the same volume of phosphate buffer. We verified that the *E. coli* strains used did not show diminished CFU after this washing procedure. Samples were distributed in 25-ml Erlenmeyer flasks (2.5 ml each), and daily-fresh hypochlorous acid was added at various concentrations from 0 to 2 mg/liter (≤100 μl). After 20 min of incubation at 25°C in the dark with gentle shaking, free chlorine was quenched by the addition of sterile sodium thiosulfate to 5×10^{-4} M. Culturable bacteria were assayed by plating on LB (43) plates after serial dilutions in cold phosphate buffer. Colonies were counted after 48 h of incubation at 37°C.

(ii) Hypochlorous acid challenge of cells collected in stationary phase. Cultures were treated after 4 days of growth. After washing, cells were resuspended in phosphate buffer at 5×10^7 bacteria per ml and treated as described above.

(iii) Adaptation experiments. To approach experimental conditions described in the literature, adaptation experiments were performed at 37°C. Cells grown in LB broth at 37°C to an optical density of 0.2 at 600 nm were washed twice and resuspended in 0.05 M phosphate buffer (pH 7.1) to which was added 30 μM H_2O_2 or 0.3 mg of HOCl per liter. After 60 min of incubation with gentle shaking at 37°C, cells were challenged with either HOCl at 2 mg/liter or H_2O_2 at 10 mM. Samples were taken at intervals. Reactions were stopped by adding sodium thiosulfate (5×10^{-4} M) or catalase (2,000 U), respectively. Results in figures are representative of experiments that were repeated at least four times.

RESULTS

A major difficulty encountered in studying the effects of HOCl on bacteria is that HOCl reacts with many compounds found in growth media, leading to production of highly reactive species. For instance, HOCl reacts with NH_4^+ and with organic amines, leading to production of monochloramine and chloramines, respectively (50). Thus, studies on intended to analyze the effect of exposure to HOCl are instead studies on effects of not properly characterized HOCl derivatives. We therefore chose for this study an experimental design in which bacteria are challenged with HOCl in phosphate buffer, which does not produce toxic derivatives of HOCl, permitting well-controlled and reproducible experimental conditions. Further, to approach conditions encountered in water distribution pipes, bacteria were grown at 25°C in minimal medium and challenged at 25°C with concentrations of HOCl in the same range as used in water treatment. The doubling time in these conditions was 4 h. Cultures referred as stationary phase had stopped growing after 60 h. Treatments were done on cells collected in stationary or exponential phase. Washing and centrifugations were done at 4°C, to avoid protein synthesis. Counting of cells before and after this procedure never resulted in a loss of viability, and control experiment showed that cells stopped in exponential phase and resuspended in growth medium immediately started growing again at a similar rate (data not shown). Further, in all experiments described below, the untreated sample was plated before and after 20 min in phosphate buffer in duplicate. No loss in colony counts was observed, whatever the mutant used, showing that the conditions of challenge have no direct effect on survival.

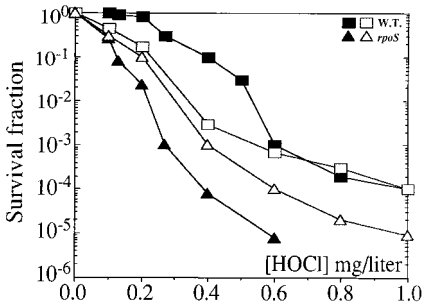

FIG. 1. Sensitivity of an *rpoS* mutant to HOCl challenge. Cultures of wild-type (W.T.) and *rpoS*::Tn*10* strains stopped in stationary (closed symbols) and exponential (open symbols) growth phases were challenged as described in Materials and Methods.

σ^s-dependent resistance to HOCl challenge. When equal concentrations of cells stopped in stationary and exponential phases were challenged with HOCl, stationary-phase cells showed higher resistance for HOCl concentrations up to 0.5 mg/liter (Fig. 1). At higher concentrations, killing rates were similar for the two cultures.

Induction in stationary phase of numerous stress resistance genes has been shown to depend on the σ^s factor, encoded by the *rpoS* gene. We assayed whether the higher HOCl resistance in stationary phase was σ^s dependent. A drastic increase in sensitivity to HOCl was observed in the *rpoS* mutant (Fig. 1). A slight sensitivity of *rpoS* was observed when cells were stopped in exponential phase, indicating some *rpoS* expression in those challenge conditions. This did not seem to result from induction of *rpoS* by cold shock during the washing procedure, since *rpoS*$^+$ and *rpoS* strains harvested in exponential phase showed the same HOCl sensitivity when washing was done at 25°C (data not shown). Interestingly, survival of the *rpoS* mutant to HOCl challenge was significantly higher when cells

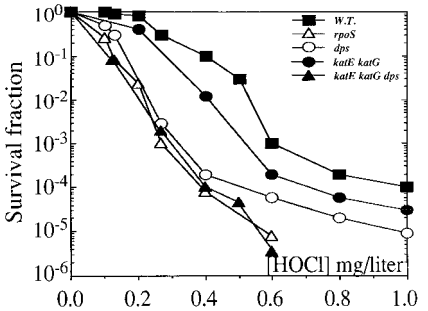

FIG. 2. Sensitivity of *dps*, *katE*, and *katG* mutants to HOCl challenge. Cultures of wild-type (W.T.), *dps*::*kan*, *katE*::Tn*10* *katG*::Tn*10*, *rpoS*::Tn*10*, and *katE*::Tn*10* *katG*::Tn*10* *dps*::*kan* strains were challenged in stationary phase.

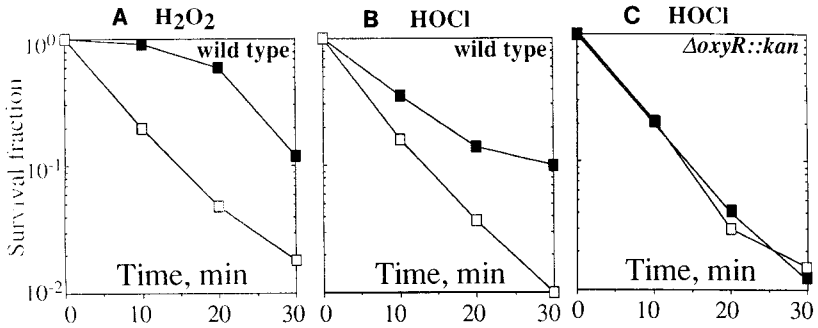

FIG. 3. Effect of H_2O_2 pretreatment on killing by HOCl. Cultures stopped in exponential phase were pretreated with 30 μM H_2O_2 and challenged with 10 mM H_2O_2 or 2 mg of HOCl per ml as described in Materials and Methods. Closed symbols, pretreated cells; open symbols, no pretreatment.

were stopped in exponential phase than when they were stopped in stationary phase, suggesting that σ^s-independent resistance mechanisms exist in exponential phase and are replaced in stationary phase by σ^s-dependent protection.

Role of Dps protein and catalases in the σ^s-dependent resistance to HOCl. The ability of HOCl and hydrogen peroxide to generate common reactive oxidative species in vitro led us to test whether, among the numerous functions controlled by σ^s, those which protect against hydrogen peroxide also participate in the σ^s-dependent protection against HOCl. Catalases (HphI and HphII) and the Dps protein, all involved in resistance to H_2O_2, are induced in stationary phase under σ^s control (5, 26, 35). The *dps* mutant showed high sensitivity to HOCl, and the protective effect of *dps* expression was markedly greater at low concentrations (up to 0.6 mg/liter), where it almost completely accounted for σ^s-mediated protection (Fig. 2). Single mutations in *katE* or *katG* had no detectable effect (data not shown), while the double mutant was slightly sensitive to HOCl. σ^s-dependent resistance was completely abolished in the *dps katE katG* triple mutant, suggesting that sensitivity was essentially due to defective expression of Dps and catalases (Fig. 2). The lack of catalases had no effect on sensitivity to HOCl on cells arrested in exponential phase, and the *dps* mutant was only weakly sensitive, behaving like the *rpoS* mutant (not shown).

Effect of H_2O_2 pretreatment on resistance to HOCl: role of the *oxyR* regulon. Since resistance to HOCl in stationary phase depends on genes involved in resistance to H_2O_2, we tested whether the adaptive response to hydrogen peroxide, governed by *oxyR*, protected the cells against HOCl. To assay whether induction of the *oxyR* regulon by H_2O_2 pretreatment occurs in conditions allowing further HOCl challenge, we verified that pretreatment with H_2O_2 performed in phosphate buffer at 37°C allows induction of resistance to H_2O_2 (Fig. 3A). The same pretreatment resulted in resistance to killing by HOCl for the wild-type strain but not for the *oxyR* defective mutant (Fig. 3B and C), showing that induction of the *oxyR* regulon by H_2O_2 protects against HOCl. It is interesting that although protection by H_2O_2 pretreatment was observed both for H_2O_2 and HOCl, the killing curves of adapted cells after the challenge were clearly different. This presumably reflects different kinds of damage promoted by the two reactants.

A constitutive *oxyR2* mutant showed higher resistance to HOCl than the wild type (Fig. 4A). In an attempt to determine which functions were responsible for the increased *oxyR*-dependent resistance, mutations in genes from the *oxyR* regulon were introduced into the *oxyR2* strain. Mutations in *katG*, *ahp*, and *dps* did not reduce the resistance of the *oxyR2* mutant (Fig. 4B).

HOCl pretreatment induces OxyR-independent resistance to H_2O_2. Since induction of the *oxyR* regulon conferred resistance to HOCl, we wondered whether, conversely, nonlethal doses of HOCl could induce *oxyR* regulon. We thus examined the effect of pretreatment with low concentrations of HOCl on H_2O_2 resistance. A set of preliminary experiments (not shown) indicated that HOCl-pretreated cells showed increased resistance to an H_2O_2 challenge for a narrow range of HOCl concentrations, with a maximum around 0.3 mg/liter. The same protection was induced in the Δ*oxyR*::*kan* strain (Fig. 5), demonstrating that protection was not mediated by activation of the *oxyR* regulon. HOCl-induced H_2O_2 resistance was also observed in an *rpoS* mutant (Fig. 5C), showing that this protection was not mediated by *rpoS* induction. Protection against HOCl stress could not be observed in these conditions (see above).

Effects of intracellular iron overload on killing by HOCl. It has been shown that in vivo, iron exacerbates the production of DNA-damaging oxidative species via the Fenton reaction (53). In a *fur* mutant, deregulation of iron homeostasis results in an intracellular iron overload, enhancing the Fenton reaction, and consequently *fur* mutants show increased sensitivity to H_2O_2. When challenged with HOCl, *fur* mutants were more sensitive than the wild type and the lethal effect increased with higher HOCl concentrations (Fig. 6A and B). This finding suggested the occurrence of a Fenton-type reaction, enhanced by iron overload.

Sensitivity to HOCl of mutants deficient in recombination repair. To test whether exposure to HOCl leads to DNA dam-

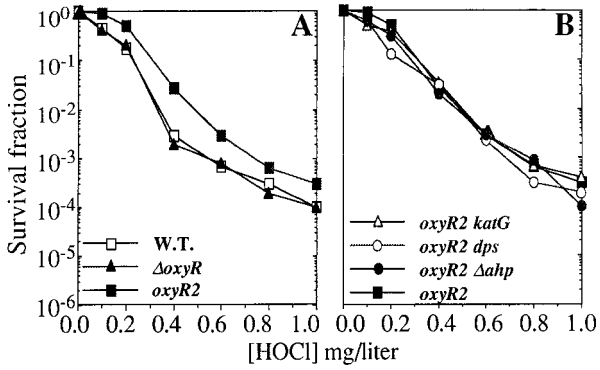

FIG. 4. Killing by HOCl in *oxyR2* constitutive mutants. Cultures stopped in exponential growth phase were treated with HOCl. (A) Wild-type (W.T.) *oxyR2* (constitutive) and Δ*oxyR*::*kan* strains; (B) *oxrR2*, *oxyR2 dps*::*kan*, *oxyR2 katG*::Tn*10*, and *oxyR2* Δ*ahp*::*kan* strains.

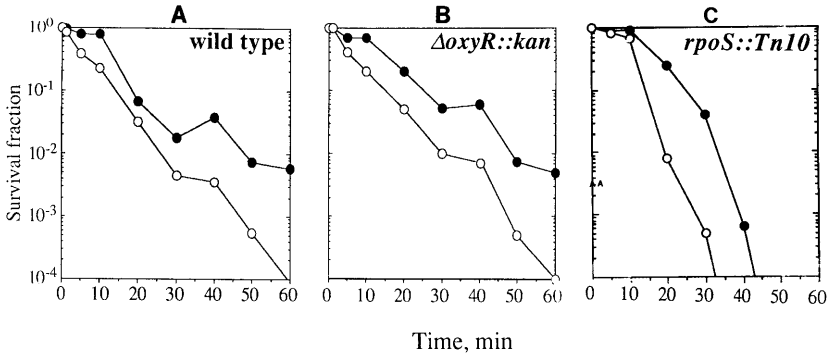

FIG. 5. Effect of HOCl pretreatment on killing by H_2O_2. Cultures stopped in exponential phase were pretreated by 0.3 mg of HOCl per liter and challenged with 10 mM H_2O_2. Closed symbols, pretreated cells; open symbols, no pretreatment.

age, we examined the effect of an HOCl challenge on mutants deficient in homologous recombination and thus unable to repair DNA strand breaks (33). A stationary culture of *recA* strain showed the same sensitivity as the wild type to low concentrations of HOCl (0.1 to 0.6 mg/liter), although at higher concentrations it became significantly more sensitive (Fig. 7A). In contrast, the *recA dps* double mutant showed drastic sensitivity, even at low HOCl concentrations, compared with the *dps* mutant. A *recB dps* strain behaved like the *recA dps* strain, while a *lexA*(Ind$^-$) *dps* strain showed sensitivity similar to that of the *dps* strain (data not shown), indicating that the hypersensitivity in the *recA dps* strain was due to its deficiency in homologous recombination rather than to its inability to induce the SOS response, the other RecA function (33). A similar effect of *recA* mutation was observed when cultures were stopped in exponential phase (Fig. 7B).

DISCUSSION

The bactericidal effect of HOCl is well recognized and widely exploited, but its mechanism of action is still poorly characterized. HOCl is an oxidative agent which can produce the same reactive oxidative species as H_2O_2, but in contrast to H_2O_2, it does not readily diffuse into cells and reacts differentially with macromolecules (3). It can, however, give rise to still reactive and much more diffusible species, such as chloramine. What is the nature of the lethal lesions? Do bacteria possess defense functions which protect them against HOCl as do defense mechanisms against other oxidative stresses? The aim of our study was to shed light on these questions.

We show that the resistance of *E. coli* to HOCl is largely mediated by genes involved in H_2O_2 resistance or induced by H_2O_2 stress, supporting the idea that similar reactive oxygen species are generated in vivo by both reactants. In stationary phase, resistance is mediated by σ^s and appears to be due to the DNA-binding protein Dps and to a lesser extent to catalases, while cells arrested in exponential phase show an *oxyR*-dependent resistance. We have not yet identified the OxyR-dependent function(s) involved in this resistance, but single mutations in *katG*, *ahp*, or *dps* did not suppress the increased resistance of *oxyR2*. The extremely high levels of HPI catalase, alkyl hydroxide reductase, and Dps in the *oxyR2* mutant may overhelm the effects of a single mutation. Alternatively, products of genes directly controlled by OxyR or genes controlled indirectly via OxyR activation of *oxyS* transcription (47) may be responsible for this *oxyR2* phenotype. Surprisingly, the Δ*oxyR* mutant was not more sensitive than wild type, suggesting that the levels of the protective function are not significantly different in uninduced *oxyR*$^+$ and Δ*oxyR* strains.

General functions could easily protect against both H_2O_2 and HOCl, but it was puzzling that a specific enzyme like catalase could protect against HOCl. In vitro reactions between H_2O_2 and HOCl or chloramines generating singlet oxygen, have been described (22, 23), and they may occur in vivo. Furthermore, it cannot be excluded that catalases acts on HOCl or its derivatives. Alternatively (or in addition), there could be a synergistic effect between damage produced by HOCl or derivatives and by the increased concentration of H_2O_2 in catalase-deficient mutants.

Pretreatment with a low concentration of HOCl protected cells from H_2O_2. Surprisingly, this mechanism was independent of OxyR. If in fact HOCl is unable to activate the *oxyR* regulon, this could explain why the wild-type strain does not show higher resistance to HOCl than the Δ*oxyR*::*kan* mutant. The HOCl-induced H_2O_2 resistance was also not dependent on σ^s. Although the *rpoS* mutant was more sensitive to H_2O_2 than the wild type, pretreated strains showed levels of similar protection (1 to 1.5 orders of magnitude). The peculiar form of the killing curve by H_2O_2 after HOCl pretreatment might reflect several different events; further work is required to unravel them. This HOCl-induced protection against H_2O_2 suggested that cells could adapt to HOCl. However, while HOCl pretreatment provided protection against H_2O_2, we were unable, under the same experimental conditions, to obtain clear protection against a challenge with higher concentrations of HOCl. This failure may be related to the phenom-

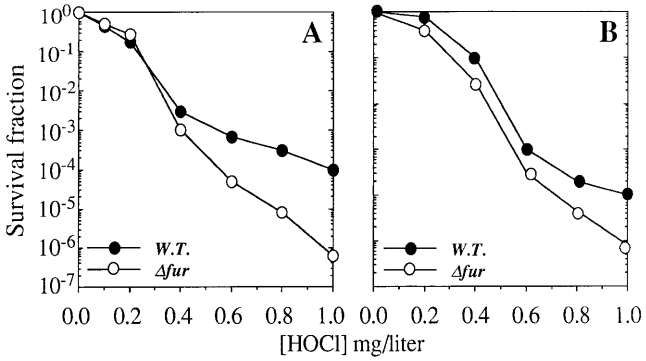

FIG. 6. Sensitivity of a *fur* mutant to killing by HOCl. Wild-type (W.T.) and Δ*fur*::*kan* cultures stopped in exponential (A) and stationary (B) growth phases were challenged with HOCl.

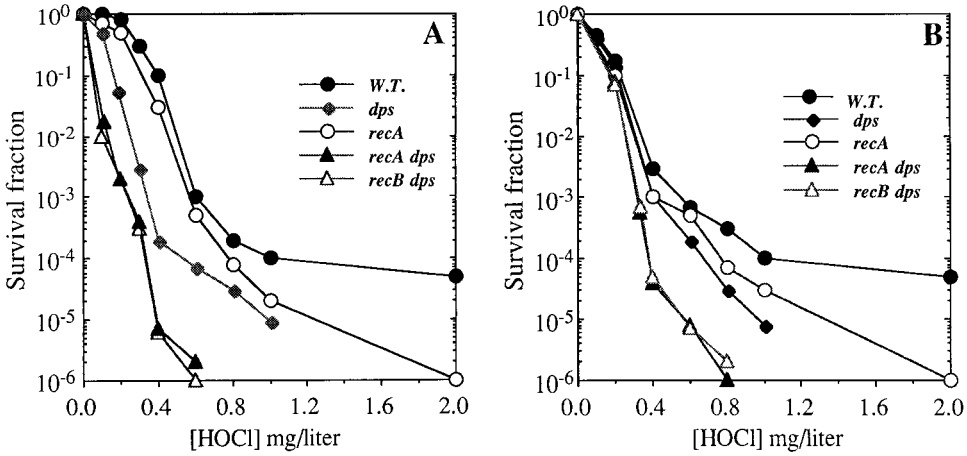

FIG. 7. Effect of deficiency in DNA homologous recombination on killing by HOCl. Cultures of wild-type (W.T.), Δ*recA*, *dps*::*kan*, Δ*recA dps*::*kan*, and *recB*::Tn*10 dps*::*kan* strains stopped in stationary (A) and exponential (B) phases were challenged with HOCl.

enon of HOCl consumption by bacteria and buffer and the fact that the HOCl concentration used for pretreatment was not negligible compared with the challenge concentration. Indeed, killing by HOCl implied that HOCl or derivatives can reach vital targets. Numerous cellular components likely react with HOCl during pretreatment with sublethal HOCl concentrations. Thus, when pretreated bacteria are challenged with higher HOCl concentrations, part of the ability of compounds to scavenge HOCl is presumably exhausted, and the kinetics of HOCl consumption is slower in pretreated cells than in unpretreated cells, complicating the comparison of killing curves.

Our data show that common protective defenses are used against H_2O_2 and HOCl. However several differences between these compounds are worth pointing out. (i) For the same concentration, killing is more drastic with HOCl than with H_2O_2 (12); challenges with H_2O_2 range from 1 to 20 mM and from 5 to 100 μM with HOCl. (ii) Candeias et al. (12) have shown that in vitro, production of hydroxyl radicals in a Fenton-type reaction is 3 orders of magnitude faster with HOCl than with H_2O_2. (iii) Reactant fate in the cell is completely different. H_2O_2, because it is poorly reactive, diffuses well into the cell but is consumed by catalases (39). HOCl reacts with many components and is unlikely to reach the DNA. DNA damage is probably caused by derivatives such as chloramine that are less reactive and thus more diffusible and able to reach the DNA more efficiently.

The increased sensitivity to HOCl of the *fur* mutant, with its iron overload, suggests that exposure to HOCl leads to production of hydroxyl radicals via a Fenton-type reaction. The lethality of these highly reactive free radicals is principally via DNA damage (24, 38).

The sensitivity of DNA repair mutants (*recA* and *recB*) to HOCl showed that it or a derivative can indeed attack DNA. In wild-type cells, significant protection is provided by the conjugate action of Dps protein and recombinational repair. This could be consistent with hypothesis that Dps, which has been shown in vitro to coat DNA (4), reduces access to DNA of damaging species.

The effects of *recA* mutation were similar when cells were arrested in exponential phase and in stationary phase, and nothing comparable to the mode 1 killing by H_2O_2 could be observed in our challenge conditions. This might be related to the difficulties encountered in attempts to study effects of HOCl on cells in exponential phase, since the reactivity of HOCl with growth media does not allow challenge of cells in growing medium. Indeed, when a *recA* strain was treated with peroxide in our challenge conditions, the mode 1 killing (not expressed in starvation conditions [25]) was reduced but not completely suppressed (unpublished data). But the absence of *recA* sensitivity at low HOCl concentrations might also indicate that DNA damage due to the Fenton reaction occurs only at higher doses. The increased sensitivity of the *fur* mutant at higher concentrations is consistent with this observation.

The results of our study suggest that exposure to HOCl causes lethal DNA damage. Other DNA damage may also occur. Mutagenesis upon HOCl exposure has been investigated in studies using various tester strains of *Salmonella typhimurium* (51, 55), with contradictory results. Chloramines were reported to be mutagenic in *S. typhimurium* and in *Bacillus subtilis* (45, 51). Epidemiological studies concluded that there is a relationship between an increased risk of bladder cancer (40) and colorectal cancer (18) and drinking chlorinated water. Whether the low HOCl concentrations used in drinking water can, in certain conditions, be mutagenic remains a question of great interest and is currently under study.

In conclusion, we have shown that exposure to HOCl can cause lethal DNA damage. There are functions that protect from killing by HOCl which all act to protect against H_2O_2 but do so in different manners. Furthermore exposure to sublethal concentrations of HOCl induces protection against H_2O_2, suggesting an adaptive response to HOCl exposure.

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