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Escherichia coli exhibits negative chemotaxis in gradients of hydrogen peroxide, hypochlorite, and *N*-chlorotaurine: Products of the respiratory burst of phagocytic cells

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ABSTRACT ***Escherichia coli* can respond to gradients of specific compounds, moving up gradients of attractants and down gradients of repellents. Stimulated phagocytic leukocytes produce H_2O_2, OCl^-, and *N*-chlorotaurine in a response termed the respiratory burst. *E. coli* is actively repelled by these compounds. Catalase in the suspending medium eliminated the effect of H_2O_2. Repulsion by H_2O_2 could be demonstrated with 1 μM H_2O_2, which is far below the level that caused overt toxicity. Strains with defects in the biosynthesis of glutathione or lacking hydroperoxidases I and II retained this response to H_2O_2, and 2.0 mM CN^- did not interfere with it. Mutants with defects in any one of the four known methyl-accepting chemotaxis proteins also retained the ability to respond to H_2O_2, but a "gutted" mutant that was deleted for all four methyl-accepting chemotaxis proteins, as well as for CheA, CheW, CheR, CheB, CheY, and CheZ, did not respond to H_2O_2. Hypochlorite and *N*-chlorotaurine were also strongly repellent. Chemotaxis down gradients of H_2O_2, OCl^-, and *N*-chlorotaurine may contribute to the survival of commensal or pathogenic microorganisms.**

Escherichia coli is capable of chemotaxis, moving up gradients of attractants and down gradients of repellents (1–4). It must be assumed that these complex responses (5–7) have evolved because they have survival value. Thus they allow the cells to seek out nutrients, as well as optimal conditions of pH, osmolarity, and oxygen concentration, and to avoid noxious substances and crowding.

Microorganisms that often live within animals are subject to phagocytic killing by host leukocytes and would be well served by being able to sense and move down gradients of compounds produced by activated phagocytes. The primary product of the respiratory burst, which occurs upon excitation of such phagocytes, is O_2^- (8, 9). The O_2^- would be converted to H_2O_2 by the dismutation reactions, both spontaneous (10) and enzyme-catalyzed (11). Myeloperoxidase would then use the H_2O_2 to oxidize Cl^- to OCl^- (12), and OCl^-, in turn, would rapidly convert taurine, another product of the phagocytic cells, to *N*-chlorotaurine (13, 14).

It thus seemed reasonable to expect *E. coli* to be repelled by gradients of H_2O_2, OCl^-, and *N*-chlorotaurine. There are, moreover, data that can be interpreted in terms of a repellent effect of H_2O_2. Thus high-intensity light, in the range of 350–450 nm, was reported to cause a tumbling response in *E. coli* (15). This could well have been due to production of H_2O_2 by a photochemical process similar to that involved in activity staining for superoxide dismutase on gels (16). More recently, a strain of *E. coli*, which accumulates protoporphyrin IX, was reported to exhibit a tumbling response to blue light (17). In this case, protoporphyrin IX probably photosensitized the production of H_2O_2. Chemotaxis away from H_2O_2 has been reported in the protozoan *Trichomonas vaginalis* (18).

We now report that *E. coli* is repelled by H_2O_2, OCl^-, and *N*-chlorotaurine.

MATERIALS AND METHODS

Chemicals. EDTA, NaH_2PO_4, Na_2HPO_4, and H_2O_2 were obtained from Mallinckrodt. NaOCl was purchased from the Aldrich, and catalase was a product of Boehringer Mannheim. Riboflavin was obtained from Eastman Chemical Products (Kingsport, TN), and *N,N,N′,N′*-tetramethylethylenediamine (TEMED) was obtained from Bio-Rad. The fluorescent dye Hoechst 33258 (2′-[4-hydroxyphenyl]-5-[4-methyl-1-piperazinyl]-2,5′-bi-1H-benzimidazole)·3HCl was from Sigma.

NaOCl concentration was determined using $\varepsilon_{292} = 350$ $M^{-1}\cdot cm^{-1}$ (19). H_2O_2 concentration was determined using $\varepsilon_{240} = 43.5$ $M^{-1}\cdot cm^{-1}$ (20).

Strains. All strains are derivatives of *E. coli* K-12. If not otherwise indicated, all experiments were performed with the wild-type chemotaxis strain AW405 (see Table 1).

Procedures. Bacteria were grown in LB medium and washed and resuspended in taxis buffer [10^{-2} M potassium phosphate buffer (pH 7.0) containing 10^{-4} M EDTA] as described by Adler (29). For the chemical-in-plug method (4), plugs were prepared by adding a small volume of the concentrated chemical to 2% liquid agar in taxis buffer cooled to just above the point of solidification.

For photochemical generation of H_2O_2, plugs were prepared by mixing 1 ml of riboflavin solution (1.0 mg/ml) with 9 ml of liquid agar containing 2% agar in taxis buffer with or without 100 μl of TEMED (final concentration in plug = 68 mM) or 70 or 100 μl of 2-mercaptoethanol (final concentration in plug = 100 or 142 mM). Plugs containing only TEMED, 2-mercaptoethanol, or riboflavin were prepared as controls. Plates were illuminated with 40-W fluorescence sources above and below the plate.

For all chemical-in-plug experiments, the 2% agar, containing the respective chemical, was allowed to solidify for 2 hr and was used the same day.

All chemical-in-pond experiments were performed as described by Tso and Adler (4). When cell number was calculated on the basis of the DNA assay (30), three capillaries were pooled in 1 ml of phosphate–saline (0.05 M sodium phosphate/2.0 M NaCl) buffer, pH 7.4, containing 0.1 μg of Hoechst 33258 per ml. In this case, cell suspensions of higher density were used (OD_{590} = 0.6–0.8 nm). The cells were sonicated for 30 sec, and fluorescence was measured. A standard curve was prepared by measuring the fluorescence of known cell numbers. However, the data are presented in terms of fluorescence units.

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Abbreviations: TEMED, *N,N,N′,N′*-tetramethylethylenediamine; MCP, methyl-accepting chemotaxis protein.

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Table 1. Strains used in this study.

Mutant	Strain	Ref.
Receptor mutants		
tsr (MCP I$^-$)	AW518	21
tar (MCP II$^-$)	AW539	21
trg (MCP III$^-$)	AW701	22
tap (MCP IV$^-$)	RP3525	23
Wild type	AW405	21
Gutted	HCB437	24
Other mutants		
cat$^-$	UM1	25, 26
Parental	GSH7	25, 26
gsh$^-$	JTG10	27
Parental	AB1157	28

MCP, methyl-accepting chemotaxis protein.

RESULTS

Hydrogen Peroxide. When the chemical-in-plug method (4) was used with rapidly motile mid-logarithmic phase cells (OD_{590} = 0.6–0.8), clear zones formed in response to H_2O_2 in the plug, and these zones increased in width as the concentration of H_2O_2 was raised from 1 mM to 32 mM (data not shown). It was noted that the diameter of the clear zones was inversely related to the density of the cell suspension used, probably because of consumption of the H_2O_2 by the hydroperoxidases in the cells. When this experiment was repeated with less motile late-logarithmic phase cells, concentric rings developed around the plugs containing high concentrations of H_2O_2, as shown by plugs E and F in Fig. 1. This probably reflects inhibition of motility by H_2O_2. Thus cells adjacent to the plug were so affected, whereas cells at some distance were at first repelled and then, in turn, inhibited by the expanding gradient of H_2O_2. The rapidly motile mid-logarithmic phase cells could stay ahead of the expanding gradient of H_2O_2, whereas the less motile late-logarithmic phase cells could not. When catalase was added to the cell suspension surrounding the plug, to a concentration of 160–320 units/ml, it eliminated the response to H_2O_2.

A strain of *E. coli* defective in both hydroperoxidases I and II (UM1; refs. 25 and 26) retained the ability to flee H_2O_2, and a strain unable to synthesize GSH (JTG10) did likewise (27). It should be noted that UM1 and JTG-10, as well as their parental strains glutathione 7 and AB1157, were of lower motility than was AW405. *E. coli* (AW405) cells that had been grown anaerobically in LB medium and then transferred to aerobic chemotaxis buffer with 0.2% glucose were unaffected by cyanide (31, 32). Chemotaxis away from H_2O_2 in such *E. coli*

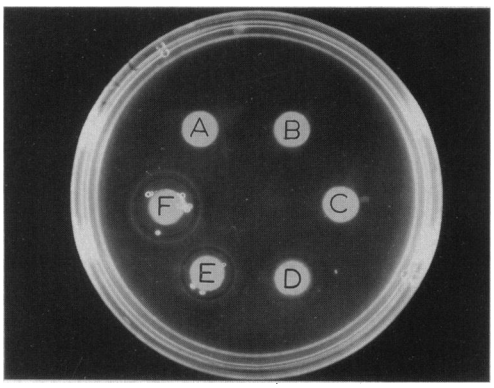

FIG. 1. Effect of H_2O_2-in-plug on late-logarithmic *E. coli*. *E. coli* AW405 was grown in LB medium to OD_{590} = 1.66 and washed and suspended in 20 ml of 0.3% agar in taxis buffer. The plugs were placed, and 1 hr later the 9-cm diameter plates were photographed. The plugs contained the following concentrations of H_2O_2: A, 0.0 mM; B, 1.0 mM; C, 4.0 mM; D, 8.0 mM; E, 16 mM; and F, 32 mM in taxis buffer.

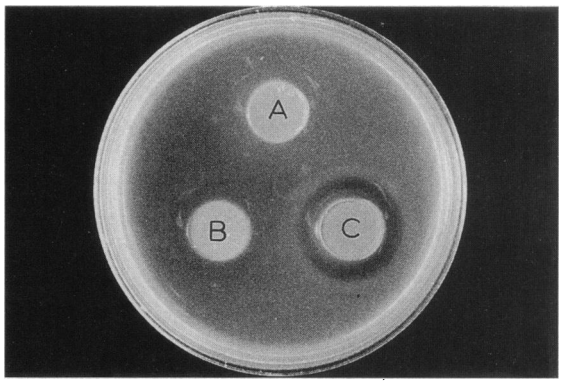

FIG. 2. Photochemical source of H_2O_2-in-plug repels *E. coli*. *E. coli* AW405, which was grown to OD_{590} = 0.65, washed, and suspended in 0.5 ml of taxis buffer, was mixed with 10 ml of 0.3% agar in taxis buffer and poured into the 5.4-cm Petri dishes. Plugs were then placed, and the plates were illuminated for 30 min. The plugs contained the following compounds: A, 0.1 mg of riboflavin per ml; B, 100 mM 2-mercaptoethanol; and C, 0.1 mg of riboflavin per ml with 100 mM 2-mercaptoethanol.

was not inhibited by 2 mM CN^-. It follows that CN^--sensitive proteins were not involved in the response to H_2O_2. *E. coli* cells that had been grown aerobically were prevented from responding by 2 mM CN^-, presumably because they contained the CN^--sensitive cytochrome *o* in place of the CN^--resistant cytochrome *d* (32) and so could not maintain an energized state in the presence of CN^-.

O_2^- can be generated photochemically, and such a photochemical source is used for activity-staining superoxide dismutase (16). Because of the spontaneous dismutation reaction, such a photochemical source of O_2^- is also a source of H_2O_2. Riboflavin was used as the photosensitizer, and TEMED or 2-mercaptoethanol was used as the electron donor. 2-Mercaptoethanol *per se* was weakly repellent. Thus an area of lower density developed around plugs containing only 2-mercaptoethanol, but it was so lacking in clarity that its diameter could not be measured with certainty. TEMED also acted as a weak repellent, causing a small zone of clearing around TEMED-laced plugs. In contrast, when plugs contained riboflavin with TEMED or with 2-mercaptoethanol, there was an illumination-dependent appearance of a ring of clearing (Fig. 2) similar to those produced by H_2O_2, and this did not develop when catalase was present. The results shown in Fig. 2 were obtained with mid-logarithmic phase cells. When late-logarithmic phase cells were used, with the photochemical source of H_2O_2, concentric rings of turbidity and clearing developed, similar to those shown in Fig. 1, and catalase inhibited this response. These results are shown in Table 2. The

Table 2. Negative chemotaxis against photochemically generated H_2O_2

Plug content	Additional components in the soft agar	Response diameter, mm	Distance migrated, mm
2-Mercaptoethanol	None	0*	0
2-Mercaptoethanol + riboflavin	None	12 (clear zone); 16 (turbid ring); 18 (clear ring)	5
2-Mercaptoethanol + riboflavin	catalase	0 mm	0

The strain AW405 was grown to OD_{590} = 1.5. Chemical-in-plug method was used. Plates were illuminated for 45 min. Chemicals were present in the plug in the following concentrations: 2-mercaptoethanol, 142 mM; riboflavin, 0.1 mg/ml; and catalase, 1625 units in the medium. Plug diameter was 8 mm.

*A slightly visible narrow zone with lower turbidity, possibly due to osmotaxis (33), could sometimes be seen.

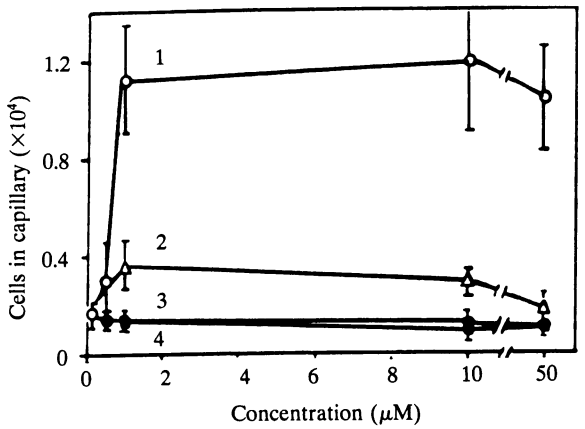

FIG. 3. Negative chemotaxis to H_2O_2 and OCl^-. The chemical-in-pond method was used, and the cells (*E. coli* AW405) in the capillary were plated for enumeration after 30 min of exposure to the indicated concentrations of H_2O_2 (line 1) or of OCl^- (line 2). In control experiments, H_2O_2 (line 3) or OCl^- (line 4) was placed both in the pond and in the capillary. The error bars denote the standard error of the mean (n = 5).

prominent clear zone around plug C in Fig. 2 was due to migration of the cells, rather than to lysis. Thus *E. coli* loses motility when grown in LB medium containing 0.4% glucose (34) or when grown to stationary phase (29). AW405 grown under either of these conditions gave neither clear zones as in Fig. 2 nor rings as in Fig. 1 in response to gradients of H_2O_2 produced as described for Figs. 1 and 2.

To obtain a quantitative measure of the repulsive effect of H_2O_2, the chemical-in-pond method (4) was used. The results shown in Fig. 3 indicate a strong repulsive effect of H_2O_2, which plateaued in the range of 1–10 μM and showed a decline at higher concentrations, probably due to inhibition of motility or to lethality at the high concentration of H_2O_2. Controls were done in which the same concentration of H_2O_2 was placed in both the pond and in the capillary, in which case there was no evidence of negative chemotaxis.

Mutants defective in MCP I, MCP II, MCP III, or MCP IV retained the ability to respond to H_2O_2, as shown in Fig. 4. A "gutted" mutant (24) that lacked MCP I–IV, as well as CheA, CheW, CheR, CheB, CheY, and CheZ, did not respond to gradients of H_2O_2.

Hypochlorite and *N*-Chlorotaurine. Hypochlorite is produced by activated neutrophils as a consequence of the action of myeloperoxidase on H_2O_2 with chloride (12). Taurine is

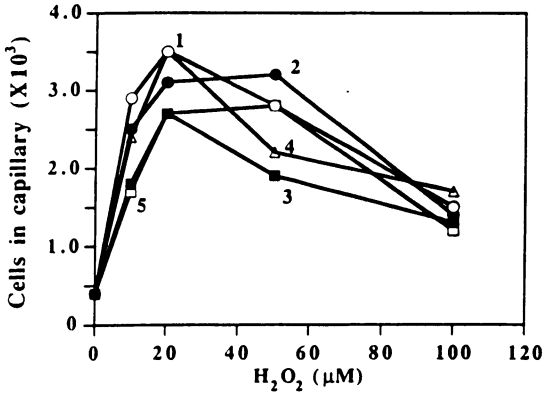

FIG. 4. MCP mutants retain the response to H_2O_2. Conditions were as described in the legend of Fig. 3, but the following *E. coli* mutants were used: line 1, parental strain (AW405); line 2, MCP I⁻ (AW518); line 3, MCP II⁻ (AW539); line 4, MCP III⁻ (AW701); and line 5, MCP IV⁻ (RP3525).

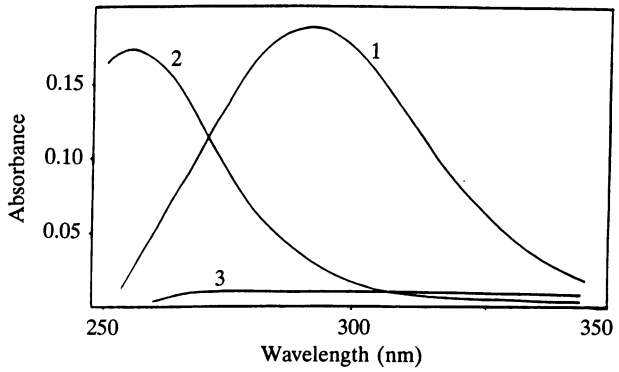

FIG. 5. Formation of *N*-chlorotaurine. Chemotaxis buffer was used as the solvent for the following compounds: line 1, 0.5 mM NaOCl; line 3, 0.5 mM taurine; and line 2, 0.5 mM NaOCl and 0.5 mM taurine; spectrum recorded 30 sec after admixture.

also released from these cells (13, 14). Fig. 5 shows that, under our conditions, hypochlorite reacts rapidly with taurine. The product of that reaction is *N*-chlorotaurine. The chemical-in-plug method demonstrated that OCl^- was a repellent but that taurine was not. A mixture of OCl^- with taurine was a more effective repellent than was OCl^-, indicating that *N*-chlorotaurine is a very effective repellent of *E. coli*.

The chemical-in-pond method was used to get a quantitative measure of the repellence of OCl^- and of *N*-chlorotaurine. With the chemical-in-pond method, we measured the cells driven into the capillary both by plating and enumeration of colonies and by fluorimetric assay of DNA. The results in Fig. 3 show that there was a repellent effect of OCl^- but it peaked

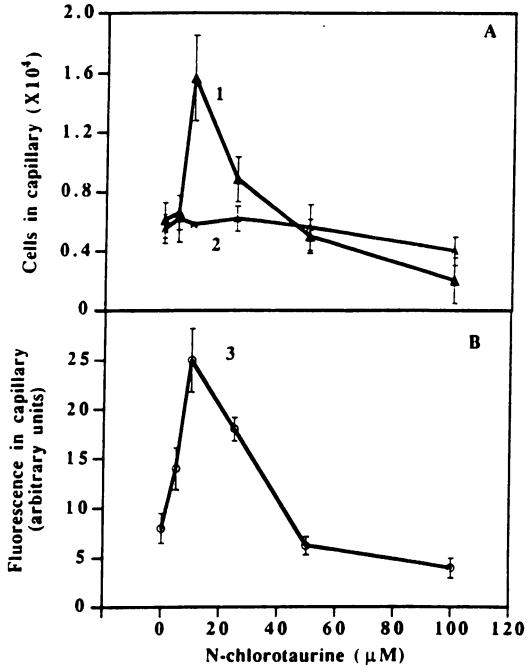

FIG. 6. Negative chemotaxis to *N*-chlorotaurine. The chemical-in-pond method was used. *N*-chlorotaurine in chemotaxis buffer was prepared 1 hr before use by admixture of 0.4 M NaOCl and 0.5 M taurine, both in taxis buffer. This stock was diluted to the desired concentration with taxis buffer. The data in *A* were obtained by plating and enumeration of the cells in the capillary, whereas the data in *B* represents DNA measured fluorimetrically. Lines 1 and 3, *N*-chlorotaurine-in-pond; line 2, *N*-chlorotaurine, in both pond and capillary. The error bars denote the standard error of the mean (n = 4).

at ≈1.0 μM and declined at higher concentration, probably due to inhibition of motility or to lethality. A more pronounced response to OCl^- was seen at lower cell density in the pond. Presumably this was due to consumption of OCl^- by the cells. Fig. 6 shows that *N*-chlorotaurine was a repellent whose effectiveness peaked at 10 μM and declined at higher concentrations. The cells that made it into the capillary remained viable as shown by the congruence of the results obtained by enumeration with those obtained by fluorimetric assay of DNA.

DISCUSSION

Neutrophils can actively seek out bacteria by moving up gradients of N-formylated peptides (35), which leak from the cells. It is now clear that the bacteria can, at the same time, flee by moving down gradients of the products of the neutrophil respiratory burst–i.e., H_2O_2, OCl^-, and *N*-chlorotaurine. Thus we have, on a microscopic scale, the familiar predator–prey relationship, with the predator pursuing and the prey fleeing. It appears possible that compounds that interfere with the signal transduction pathway mediating the negative chemotaxis we have described would constitute a new class of antibiotics.

Mutants in MCP I, MCP II, MCP III, and MCP IV retained the ability to move down gradients of H_2O_2. However, gutted mutants lacking all four MCPs (MCP I–IV), as well as CheA, CheW, CheR, CheB, CheY, and CheZ, failed to respond. This indicates that the known mechanism of chemotaxis in *E. coli* is involved in the response to H_2O_2. Evidently the mechanism involved in mediating this response to H_2O_2, OCl^-, and *N*-chlorotaurine needs to be explored.

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