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Potentiation by Sulfide of Hydrogen Peroxide-Induced Killing of *Escherichia coli*

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L-Cysteine potentiates 100-fold the hydrogen peroxide-induced killing of a growing culture of *Escherichia coli* K-12 (Berglin et al., J. Bacteriol. 152:81–88). In the present study it is shown that hydrogen sulfide is formed from L-cysteine and that sodium sulfide could substitute for L-cysteine in the potentiation of hydrogen peroxide-induced killing of *E. coli* K-12. Addition of an amino acid, L-leucine, L-valine, or L-alanine, to an L-cysteine-containing medium with a growing culture of *E. coli* K-12 inhibited hydrogen sulfide formation and the potentiation of hydrogen peroxide-induced killing. These amino acids did not inhibit hydrogen sulfide formation from L-cysteine by a cell extract, and they did not inhibit the potentiation by sulfide of hydrogen peroxide-induced killing. This indicated that the amino acids protected the culture from L-cysteine-potentiated, hydrogen peroxide-induced killing by inhibiting the transport of L-cysteine into the cell. The potentiation by sodium sulfide of hydrogen peroxide-induced killing was abolished by the metal ion chelator 2,2′-bipyridyl. This indicated that metal ions, in addition to sulfide, were involved in the killing. Toxic effects of hydrogen peroxide are often presumed to be mediated by hydroxyl radicals formed in iron-catalyzed reactions. It was demonstrated that iron sulfide was more efficient than ferrous iron in catalyzing the formation of hydroxyl radicals from hydrogen peroxide. It was suggested that hydrogen sulfide formed in polymicrobial infections may play an important role in the host defense by potentiating the antimicrobial effect of hydrogen peroxide produced by phagocytic cells.

In the host defense against bacterial infections hydrogen peroxide produced by phagocytic cells has an important role (3, 38). From hydrogen peroxide the highly bactericidal hypochlorite is formed in a myeloperoxidase-catalyzed oxidation of chloride ions (38). Hydrogen peroxide per se can also be bactericidal (9, 12, 22, 24), but the nature of the lethal injury inflicted on bacteria is not fully understood.

It is known that hydrogen peroxide induces DNA strand breaks in both bacteria and mammalian cells (12, 22, 23, 31) and that the efficacy of DNA repair of the cell is important in protecting against hydrogen peroxide-induced killing (7, 11, 22, 27, 40). Hydrogen peroxide does not cause breaks in purified DNA (14, 31), but DNA binds ferrous iron in such a way that hydroxyl radicals are formed from hydrogen peroxide (Fenton reaction) and these radicals split DNA (14, 19). The finding that metal ion chelators such as phenanthroline and bipyridyl prevent hydrogen peroxide-induced killing and DNA strand break formation both in bacteria and in mammalian cells indicates that metal ions catalyze hydrogen peroxide-induced killing and that DNA strand breaks might be significant for the killing (6, 8, 23, 29, 30). Hydrogen peroxide can also react with ferrous iron bound to proteins resulting in hydroxyl radical formation which inactivates the proteins (15, 26, 33). However, the protecting effect of bipyridyl may not exclusively be ascribed to its capacity to chelate metal ions, since this substance may also inhibit the metabolic activity of bacteria, and such inhibition decreases the toxic effects of hydrogen peroxide (35).

We have previously shown that L-cysteine potentiated 100-fold the bactericidal effect of hydrogen peroxide and DNA strand break formation in *Escherichia coli* K-12 in the presence of hydrogen peroxide (6). We now report that the potentiation of hydrogen peroxide-induced killing by L-cysteine is related to hydrogen sulfide formation from L-cysteine.

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MATERIALS AND METHODS

Microorganisms. *E. coli* K-12 strain K37, an Str^r mutant strain of W3102 (4) was obtained from the Department of Microbiology, University of Umeå. This strain was kept on a minimal medium agar in a glove box with an atmosphere of 10% hydrogen–5% carbon dioxide in nitrogen. Unless otherwise stated, all experiments were performed in this anaerobic environment. All solutions and media were kept at least for 2 days in the anaerobic box before use. The redox potential of a dilution solution (pH 7.0) then became lower than −350 mV.

Media. The minimal medium had the following composition: 40 mM potassium morpholinopropane sulfonate–KOH (MOPS; pH 7.0), 20 mM glucose, 10 mM NH_4HCO_3, 25 mM NaCl, 1 mM K_2HPO_4, 0.5 mM $MgCl_2$, 0.25 mM K_2SO_4, and 0.01 mM $FeCl_3$. The medium was filter sterilized, and $FeCl_3$ from a fresh solution was added just before use. This medium is a modification of the medium devised by Neidhardt et al. (34). The minimal agar medium contained 16 g of agar (Difco Laboratories) per liter.

To avoid hydrogen peroxide formation in the medium (9), the peptone-yeast extract-glucose agar medium was prepared from two solutions. One solution contained 20 g of tryptone (Difco), 10 g of yeast extract (Difco), and 20 g of NaCl in 1,000 ml. The other solution contained 28 g of agar (Difco) and 2 g of glucose in 1,000 ml. The two solutions were autoclaved, cooled to 50°C, mixed, and poured into plates.

The dilution solution was also prepared from two solutions. One solution contained (pH 7.0) 64 mM sodium β-glycerophosphate, 11.3 mM KCl, 147 mM NaCl, 14.1 mM Na_2HPO_4, and 14.7 mM KH_2PO_4. The other solution con-

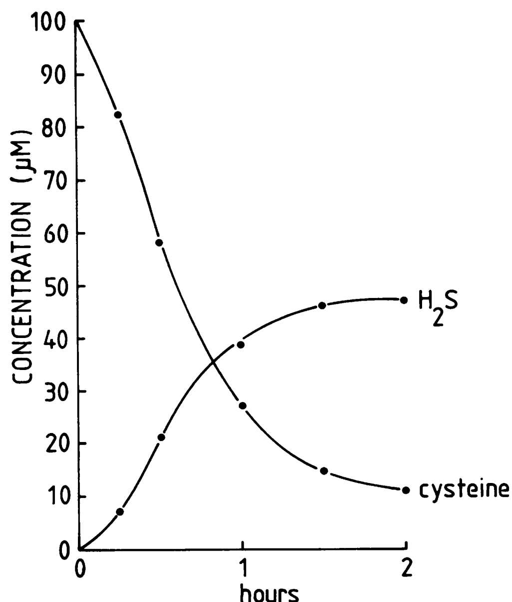

FIG. 1. Decomposition of L-cysteine and formation of hydrogen sulfide in a growing culture (6×10^7 cells ml^{-1}) of *E. coli* K-12.

tained 3.8 mM $CaCl_2$ and 1.0 mM $MgCl_2$. The two solutions were autoclaved, cooled, and pooled.

For the complex medium (TSYP), 34 g of Trypticase (BBL Microbiology Systems), 6 g of Phytone (BBL), 5 g of NaCl, and 10 g of yeast extract (Difco) in 1,000 ml of water were autoclaved, cooled, and pooled with 1,000 ml of an autoclaved and cooled solution containing 0.1 mol of K_2HPO_4 and 0.1 mol of KH_2PO_4. Just before use 60 ml of a filter-sterilized solution containing 0.01 mol of NH_4HCO_3, 0.01 mol of sodium pyruvate, and 0.055 mol of glucose was added.

Chemicals. Hydrogen peroxide (30% [wt/wt]; Perhydrol) was from E. Merck AG, Darmstadt, Federal Republic of Germany. All amino acids were in the L form. Cysteine was from Sigma Chemical Co., St. Louis, Mo. All other amino acids were from the British Drug Houses, Poole, England. Ferrous chloride (0.1 M) was dissolved in 0.1 M HCl and diluted when necessary in 1 mM HCl. Water was double distilled in quartz vessels. All other chemicals were of reagent grade.

Bactericidal effect of hydrogen peroxide. *E. coli* K-12 was grown overnight in the minimal medium. When still in the exponential growth phase, the culture was transferred to fresh medium. The generation time was then 126 ± 13 min (standard deviation). After growth in this medium for about three generations to a density of about 10^8 cells ml^{-1}, the culture was exposed to hydrogen peroxide in the presence of various compounds as indicated. The following amino acids (2.6 mM) were used: L-alanine, L-valine, L-leucine, L-threonine, L-methionine, L-histidine, L-glycine, L-isoleucine, L-phenylalanine, L-serine, L-tyrosine, L-tryptophan, L-proline, L-lysine, L-arginine, L-aspartic acid, L-glutamic acid, L-asparagine, and L-glutamine. Samples (0.1 ml) for determination of viable counts were taken from the culture before and 20 min after the addition of hydrogen peroxide. The samples were spread over the surface of peptone-yeast extract-glucose agar and incubated at 37°C overnight.

Cysteine decomposition and hydrogen sulfide formation. An exponentially growing culture of *E. coli* K-12 in the minimal medium was transferred into fresh minimal medium and grown at 37°C for three generations to a density of about 6×10^7 cells ml^{-1}, when L-cysteine was added. At various times samples were taken, and the concentrations of L-cysteine (16) and hydrogen sulfide (41) were determined.

Preparation of cell extract. An aerobic culture of *E. coli* K-12 grown overnight in TSYP medium was inoculated into 500 ml of fresh TSYP medium, and 0.05 mmol of L-cysteine was added. The culture was incubated in air at 37°C in a 1,000-ml Erlenmeyer flask on a shaker with a circular orbital motion (100 rpm). The culture was allowed to grow for four generations to a cell density of about 4×10^8 cells ml^{-1}. One hour before reaching this density another 0.05 mmol of L-cysteine was added. The cells were harvested and washed four times in a solution containing 40 mM potassium phosphate buffer (pH 6.8), 66 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 0.5 mM $MgCl_2$. The cells were then suspended in this solution to a density equivalent to 50 mg (dry weight) ml^{-1}. The cells were kept at −80°C until they were thawed and disrupted by sonic oscillation for 5 min at 4°C (200 W, 2 A). The extract was centrifuged for 30 min at $30,000 \times g$, and the supernatant fluid was dialyzed overnight in 300 times its volume of 10 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 3 mM $MgCl_2$, and 5 mM mercaptoethanol. The protein content of the cell extract was determined by the biuret method (25).

Assay of cysteine desulfhydrase activity. The cell extract was brought into the anaerobic box, and enzyme activity was assayed at 37°C in a reaction mixture (1 ml) containing 71 µmol of potassium phosphate buffer (pH 7.4), 0.5 µmol of pyridoxal 5-phosphate, and 0.5 µmol of L-cysteine. Samples were taken at various times, and the concentration of hydrogen sulfide was determined (41).

Preparation of iron sulfide. In an anaerobic environment of 10% hydrogen in nitrogen, 5 ml of 1 M MOPS buffer (pH 7.0), 5 ml of 0.75 M NaCl, 5 ml of 10 mM Na_2S, 5 ml of 10 mM ferrous chloride, and 30 ml of water were mixed. The iron sulfide was allowed to precipitate for 30 min at room temperature, and the precipitate was washed twice in water and suspended in 25 ml of water. The concentration of iron in the suspension was estimated (42) after iron sulfide had been dissolved by the addition of 1 M HCl.

Deoxyribose decomposition. In these experiments the anaerobic environment was 10% hydrogen in nitrogen. The reaction mixture contained (in 1 ml) 1 µmol of deoxyribose, 75 µmol of NaCl, 100 µmol of MOPS buffer (pH 7.0) and 1 µmol of H_2O_2. The reaction was initiated by adding various amounts of ferrous iron, sodium sulfide, or iron sulfide. After 15 min at 37°C the reaction was stopped by the addition of 1 ml of a 1% thiobarbituric acid solution (wt/vol, in 0.05 M NaOH) followed by 1.0 ml of 2.8% (wt/vol) trichloroacetic acid by the method of Gutteridge and Halliwell (18). The tubes were heated for 10 min at 100°C, and after cooling the absorbance at 532 nm was read. The same reaction mixtures without deoxyribose were used as blanks.

Statistics. Statistical analysis was performed using the paired and unpaired Student *t*-test as appropriate. All values are presented as means and standard errors of the means from at least three independent experiments.

RESULTS

L-Cysteine was decomposed by a growing culture of *E. coli* K-12 and hydrogen sulfide was formed (Fig. 1). We have previously found that the ability of L-cysteine to potentiate the hydrogen peroxide-induced killing of a growing culture of *E. coli* K-12 was greatest when L-cysteine was added to

the culture 30 to 120 min before hydrogen peroxide (6). The present finding that hydrogen sulfide was formed from L-cysteine within that time suggested that hydrogen sulfide rather than L-cysteine was responsible for the potentiation of hydrogen peroxide-induced killing. When sodium sulfide was added to the culture, it actually potentiated hydrogen peroxide-induced killing almost as efficiently as L-cysteine (Table 1).

The toxic effect of hydrogen peroxide in the presence of L-cysteine in a growing culture of *E. coli* K-12 was decreased by L-alanine ($P < 0.01$), L-valine ($P < 0.02$), L-leucine ($P < 0.001$), L-threonine ($P < 0.02$), and L-methionine ($P < 0.02$; Table 2). The potentiation of hydrogen peroxide-induced killing by sodium sulfide was not influenced by these amino acids (data not shown). No other amino acid influenced the toxic effect of hydrogen peroxide in the presence of L-cysteine.

Hydrogen sulfide formation from L-cysteine by a growing culture of *E. coli* K-12 (Table 2) was also inhibited by the amino acids L-leucine ($P < 0.01$), L-valine ($P < 0.02$), L-alanine ($P < 0.02$), L-methionine ($P < 0.04$), and L-threonine ($P < 0.05$). Addition of 2.6 mM L-histidine did not affect cysteine decomposition or hydrogen sulfide formation (Table 2).

A cell extract of *E. coli* K-12 had cysteine desulfhydrase activity, which formed from L-cysteine 1.7 ± 0.1 nmol of hydrogen sulfide min^{-1} mg of $protein^{-1}$. L-Leucine, L-valine, or L-alanine (13 mM) did not inhibit the activity of cysteine desulfhydrase in the cell extract.

In another series of experiments one of the amino acids, L-leucine, L-valine, or L-alanine, was added to growing cultures of *E. coli* K-12 30 min before the cultures were exposed to hydrogen peroxide. L-Cysteine had been added to these cultures at different times before the addition of the amino acid. The percentage of surviving cells after hydrogen peroxide exposure increased with decreasing time interval between the addition of L-cysteine and the L-amino acid (Table 3). Since these three amino acids decreased the rate of hydrogen sulfide formation from L-cysteine in the growing culture but did not interfere with the activity of cysteine desulfhydrase, these findings suggest that the amino acids interfered with the transport of L-cysteine into the cell.

The hydrogen peroxide-induced killing potentiated by L-cysteine or sodium sulfide could be overcome by the addition of the metal ion chelator 2,2'-bipyridyl to the growing culture (Table 1). The release of hydrogen sulfide from L-cysteine by the growing culture was decreased by 2,2'-bipyridyl ($P < 0.01$; Table 2). The chelator 2,2'-bipyridyl was, however, much more efficient than L-leucine ($P < 0.004$), L-alanine ($P < 0.001$), and L-valine ($P < 0.001$) in protecting against hydrogen peroxide-induced killing (Table 2). This indicates that metal ions might be involved in the killing and that the lethal injury is caused by hydroxyl radicals formed from hydrogen peroxide in the presence of iron (20).

The formation of hydroxyl radicals from hydrogen peroxide is presumptively demonstrated by the capacity of these radicals to decompose deoxyribose into thiobarbituric acid-reactive products (19). When deoxyribose was exposed to hydrogen peroxide in the presence of ferrous iron, sodium sulfide, or iron sulfide, iron sulfide was more efficient than ferrous iron ($P < 0.01$) and sodium sulfide ($P < 0.001$) in catalyzing the decomposition of deoxyribose (Fig. 2, Table 4). The chelator 2,2'-bipyridyl abolished not only the effects of ferrous iron and iron sulfide but also that of sodium sulfide (Table 4). This indicates that the weak effect of sodium

TABLE 1. Effect of L-cysteine and sodium sulfide on hydrogen peroxide toxicity in growing cultures of *E. coli* K-12

Additions[a]	Survivors (%)[b]
H_2O_2 (10 mM)	1 ± 0.4
H_2O_2 (0.1 mM)	117 ± 3.2
H_2O_2 (0.1 mM) + 0.1 mM L-cysteine	1 ± 0.2
H_2O_2 (0.1 mM) + 0.1 mM L-cysteine + 0.1 mM 2,2'-bipyridyl	95 ± 5.6
H_2O_2 (0.1 mM) + 0.1 mM sodium sulfide	3 ± 1.7
H_2O_2 (0.1 mM) + 0.1 mM sodium sulfide + 0.1 mM 2,2'-bipyridyl	74 ± 4.3
None	110 ± 2.3

[a] L-Cysteine in combination with 2,2'-bipyridyl was added to the culture 30 min before the culture was exposed to hydrogen peroxide. Sodium sulfide or sodium sulfide in combination with 2,2'-bipyridyl was added 10 min before the culture was exposed to hydrogen peroxide.

[b] The percentage of surviving cells 20 min after the culture was exposed to hydrogen peroxide is given. L-Cysteine and sodium sulfide by themselves did not affect the viability of the cells.

TABLE 2. Formation of hydrogen sulfide from 0.1 mM L-cysteine in the presence of various amino acids in growing cultures of *E. coli* K-12 and the toxicity of hydrogen peroxide in these cultures

Additions[a]	Hydrogen sulfide formed (nmol, ml^{-1} 30 min^{-1})	L-Cysteine decomposed (nmol, ml^{-1} 30 min^{-1})	Survivors (%)[b]
L-Cysteine	21 ± 1.1	44 ± 1.8	1 ± 0.2
+ L-Alanine	7 ± 0.8	27 ± 2.3	26 ± 6.7
+ L-Valine	5 ± 0.7	22 ± 1.3	16 ± 3.4
+ L-Leucine	7 ± 0.5	23 ± 0.7	45 ± 6.6
+ L-Threonine	13 ± 1.2	30 ± 1.2	9 ± 2.0
+ L-Methionine	12 ± 0.6	66 ± 2.9	6 ± 0.6
+ L-Histidine	22 ± 3.1	36 ± 1.6	0.06 ± 0.02
+ 2,2'-Bipyridyl	11 ± 0.6	35 ± 3.1	95 ± 5.6

[a] The amino acid (2.6 mM) or 2,2'-bipyridyl was added to the culture together with 0.1 mM L-cysteine, and the decrease of the L-cysteine level and the increase of the hydrogen sulfide level were determined after 30 min.

[b] In separate experiments the amino acid (2.6 mM) or 0.1 mM 2,2'-bipyridyl was added to the culture together with 0.1 mM L-cysteine to the culture, and 30 min thereafter the culture was exposed to 0.1 mM hydrogen peroxide. The percentage of surviving cells 20 min after the culture was exposed to hydrogen peroxide is given.

TABLE 3. Effect of various amino acids on hydrogen peroxide toxicity in the presence of L-cysteine, when L-cysteine was added to growing cultures of *E. coli* K-12 at various times before the amino acid

Amino acid	Survivors (%) at the following times (min) of L-cysteine addition[a]			
	30	20	10	0
L-Leucine	2 ± 0.1	6 ± 1.7	17 ± 4.5	45 ± 6.6
L-Alanine	2 ± 0.6	2 ± 1.0	12 ± 1.4	26 ± 1.4
L-Valine	3 ± 0.1	7 ± 2.2	14 ± 4.0	16 ± 3.4
None	2 ± 0.1	1 ± 0.2	1 ± 0.2	1 ± 0.2

[a] L-Cysteine (0.1 mM) was added to the culture at various times before the culture was supplied with one of the (2.6 mM) amino acids: L-leucine, L-valine, or L-alanine. The culture was exposed to 0.1 mM hydrogen peroxide 30 min after the addition of the amino acid. The percentage of surviving cells 20 min after the culture was exposed to hydrogen peroxide is given.

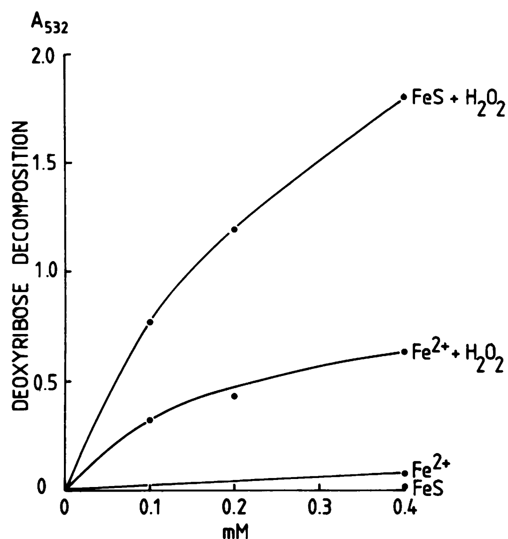

FIG. 2. Decomposition of deoxyribose measured as formation of thiobarbituric acid-reactive products (absorbance at 532 nm [A_{532}]) in reaction mixtures containing 1 mM deoxyribose, 75 mM sodium chloride, 100 mM MOPS buffer (pH 7.0), various amounts of ferrous ions or iron sulfide, and 1 mM hydrogen peroxide as indicated.

sulfide on the decomposition of deoxyribose in the presence of hydrogen peroxide is dependent on some metal ion contamination of the sodium sulfide preparation.

L-Histidine increased the hydrogen peroxide-induced killing potentiated by L-cysteine (Table 2). In fact L-histidine per se was able to potentiate the hydrogen peroxide-induced killing. The percentage of surviving cells 20 min after the exposure of a growing culture to 0.1 mM hydrogen peroxide was 3 ± 0.6 in the presence of 2.6 mM L-histidine. This effect of L-histidine was abolished by 0.1 mM 2,2'-bipyridyl. There were 72 ± 7.5% survivors 20 min after the cells were exposed to 0.1 mM hydrogen peroxide in the presence of 2.6 mM L-histidine and 0.1 mM 2,2'-bipyridyl.

DISCUSSION

A bacteriostatic effect of L-cysteine on *E. coli* has been known for a long time (39). This effect is due to an inhibition of L-isoleucine synthesis at the level of threonine deaminase (21). L-Cysteine may also have a bactericidal effect. Lethal levels of hydrogen peroxide are formed when L-cysteine is oxidized in aerated culture media (8, 36). This bactericidal effect of hydrogen peroxide is potentiated by L-cysteine (6) and has now been shown to be related to the formation of hydrogen sulfide from L-cysteine.

We have previously demonstrated killing and DNA break formation in a growing culture of *E. coli* K-12 exposed to hydrogen peroxide in the presence of L-cysteine (6). It has been suggested that the killing and DNA lesions were induced by hydroxyl radicals formed from hydrogen peroxide in a reaction catalyzed by some metal ions, possibly iron, since both killing and DNA lesions were prevented by the chelator 2,2'-bipyridyl (6), but the role of L-cysteine in this reaction was obscure. The present finding that hydrogen sulfide was formed from L-cysteine by the culture shed new light on the problem.

The fact that the effect of sulfide was abolished by the metal ion chelator 2,2'-bipyridyl might indicate that some metal ion was involved in the hydrogen peroxide-induced killing. This chelator also decreased the formation of hydrogen sulfide from L-cysteine, but this could hardly explain the protection against hydrogen peroxide toxicity offered by 2,2'-bipyridyl, since the amino acids L-leucine, L-valine, and L-alanine were equally efficient in inhibiting hydrogen sulfide formation but less efficient in protecting against hydrogen peroxide toxicity. Since iron is chelated by 2,2'-bipyridyl, it was tested whether iron sulfide catalyzed the formation of hydroxyl radicals from hydrogen peroxide. It was found that iron sulfide was actually more efficient than ferrous iron in catalyzing the reaction. It has recently been shown that iron-catalyzed formation of hydroxyl radicals from hydrogen peroxide (Fenton reaction) requires the availability of at least one iron coordination site that is free or occupied by a readily dissociable ligand such as water (17). In iron sulfide each iron atom is surrounded octahedrally by six sulfur atoms and also has two near iron neighbors which are coplanar with four of the sulfur atoms (10). The sulfide is, however, nonstoichiometric, with metal-metal bonding and with some of the iron positions in the lattice vacant in a random way (10). This structure may leave free coordination sites of iron, which may catalyze the formation of hydroxyl radicals from hydrogen peroxide.

Most of the iron of the cell is usually in such a form that it does not catalyze the Fenton reaction (20). It is possible, however, that some of the sulfide formed in the living cell as a result of normal metabolic activity could be bound to iron and that the iron sulfide complex may catalyze the Fenton reaction. It has recently been reported that ornithine decarboxylase in rat liver homogenates is inactivated when incubated with L-cysteine. The inactivation of the enzyme was correlated with the formation of hydrogen sulfide during incubation and with an increase in reactive ferrous iron in the homogenate (32). Hydrogen sulfide was thought to activate iron in such a way that it catalyzed the oxidation of essential thiol groups of the enzyme by oxygen. Although the interpretation of their findings was different from ours, both studies indicate that hydrogen sulfide might be involved in oxygen and hydrogen peroxide toxicity in a way that has not previously been realized.

Although the transport of L-cysteine in mammalian cells has been thoroughly elucidated (5), very little is known about the transport of L-cysteine in *E. coli* (2). The only information so far available is that the transport of L-cysteine may be coupled to a membrane-bound D-lactate dehydrogenase. This transport is inhibited by anaerobiosis and by the amino acids L-leucine, L-isoleucine, and L-valine (28). The finding of the present study that L-leucine, L-valine, and L-alanine inhibit hydrogen sulfide formation from L-cysteine by intact cells but not that by cell extracts was interpreted as

TABLE 4. Effect of 2,2'-bipyridyl on decomposition of deoxyribose induced by hydrogen peroxide in the presence of iron or sulfide or both

Addition (0.2 mM)[a]	Deoxyribose decomposition at the following concn (mM) of 2,2'-bipyridyl[b]		
	0	0.2	3.2
Ferrous iron	0.54 ± 0.03	0.41 ± 0.06	0
Iron sulfide	1.18 ± 0.03	0.13 ± 0.01	0
Sodium sulfide	0.07 ± 0.03	0	0

[a] The reaction mixture contained 1 mM deoxyribose; 75 mM sodium chloride; 100 mM MOPS buffer (pH 7.0); 1 mM hydrogen peroxide; and 0.2 mM ferrous iron, 0.2 mM iron sulfide, or 0.2 mM sodium sulfide and 2,2'-bipyridyl as indicated.

[b] The deoxyribose decomposition is expressed as the formation of thiobarbituric acid-reactive products measured at an absorbance of 532 nm.

an indication that these amino acids interfered with the transport of L-cysteine. Since our experiments were performed under anaerobic conditions, this indicates that transport systems for L-cysteine other than D-lactate-dependent transport might exist.

The finding that L-histidine potentiated hydrogen peroxide-induced killing was unexpected, since L-histidine is a very efficient hydroxyl radical scavenger (13, 33). L-Histidine, however, has high affinity for iron (1), and it is possible that L-histidine became bound to iron in such a way that the iron-catalyzed formation of hydroxyl radicals from hydrogen peroxide was facilitated. There is also the possibility that L-histidine interfered with some protection system against hydrogen peroxide.

The potentiation by sulfide of hydrogen peroxide-induced killing may be of significance in the antimicrobial host defense. In polymicrobial infections, such as periodontal disease, hydrogen sulfide is formed in large amounts (37) and may thus increase the bactericidal effect of hydrogen peroxide from the phagocytic cells. Hydrogen sulfide is, however, also known to be highly toxic to mammalian cells, and an interesting question is whether this toxicity is to some extent dependent on iron sulfide as a catalyst of hydroxyl radical formation from hydrogen peroxide in the cell.

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