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Original Contribution

EFFECTS OF MENADIONE AND HYDROGEN PEROXIDE ON GLUTATHIONE STATUS IN GROWING *ESCHERICHIA COLI*

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Abstract—Menadione (MD) and H_2O_2 caused distinct effects on glutathione status in growing *Escherichia coli*. Treatment of *E. coli* AB1157 with 1–25 mM H_2O_2 did not result in an appreciable decrease in intracellular total glutathione (reduced glutathione [GSH] + oxidized glutathione [GSSG]). Only when cells were treated with 25 mM H_2O_2 an increase in GSSG and a decrease in the GSH:GSSG ratio were observed. In cells deficient in catalase HPI, such effect was observed even at 10 mM H_2O_2. The exposure of *E. coli* AB1157 to MD caused a dose-dependent decrease in intracellular total glutathione, an increase in GSSG, and a decrease in the ratio of GSH:GSSG. In *E. coli* deficient in cytosolic superoxide dismutase activity, a decrease in total glutathione after incubation with 0.2 mM MD was not accompanied by an increase in $GSSG_{in}$, and the ratio of GSH_{in}:$GSSG_{in}$ was three times higher than in the wild-type cells. The changes in the redox status of extracellular glutathione under the action of both oxidants were similar. Although the catalase activity increased several times after exposure to both oxidants, there were little or no changes in the activity of enzymes related to glutathione metabolism. A possible role of changes in redox status of glutathione under oxidative stress is discussed. © 2000 Elsevier Science Inc.

Keywords—*Escherichia coli*, Glutathione, Menadione, Hydrogen peroxide, Free radical

INTRODUCTION

Reduced glutathione (GSH) is the most abundant and ubiquitous nonprotein thiol in eucaryotic and procaryotic cells. GSH plays an important role in protecting cells against oxidative damage, toxic compounds, radiation and heavy metals [1]. It is suggested that GSH as well as other low-molecular weight thiols may be involved in the regulation of cellular metabolism by changes in redox state of protein thiols. Formation of mixed disulfides or intramolecular disulfides can significantly modulate enzymic activity. The cellular thiol: disulfide redox balance (in particular, the ratio GSH:oxidized glutathione [GSSG]) is important parameter in processes in which mixed disulfides involved [2]. Under physiological conditions the concentration of GSSG is very low and the ratio of GSH:GSSG is high. However, in mammalian cells under conditions of oxidative stress the concentration of GSSG increase and the GSH:GSSG ratio decreases [2,3]. In these cells glutathione is an important component of the cellular defenses against damage produced by exposures to oxidants such as H_2O_2 and other reactive oxygen species [1,4].

The role of glutathione in protection of bacteria against oxidants and free radicals was investigated many fewer. It was reported that growing cells of *Escherichia coli* devoid of GSH have normal resistance to H_2O_2, cumene hydroperoxide, *tert*-butyl hydroperoxide, and paraquat [5–7]. However, nongrowing glutathione-deficient *E. coli* are more susceptible to killing by H_2O_2 and chlorine oxidants than are the GSH-replete cells [7]. It was suggested that the role of GSH in bacteria differs from its functions in mammalian cells [5,6].

Little is known about the effect of oxidants on the levels and redox status of intra- and extracellular glutathione in bacteria. In *E. coli* cells, glutathione is the main part of acid-soluble thiols [8]. It was shown that exposure of growing *E. coli* AB1157 to H_2O_2 did not change the level of acid-soluble thiols [5]. In previous study, we showed that treatment of growing *E. coli* K12 with 1 mM H_2O_2 increased the level of intracellular acid-soluble thiols [9]. Other workers reported that exposure of *E. coli*

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Table 1. Bacterial Strains Used in this Study

Strain	Genotype	Source or reference
E. coli		
AB1157	*thr-1 leuB6 thi-1 arg-E3 his-4 proA2 tsx-33 supE44 lacY1 galK2 ara-14 xyl-5 mtl-1 rpsL*	CGSC[a]
QC909	*F⁻Δ(lac)4169 rpsL Φ(sodA::Mu dPR13) Φ(sodB::kan) Δ1-2 25*	22
JHC1092	*F⁻Δ(lac4169 rpsL Δ(soxR zjc-2205) zjc-2204::Tn10km)*	10
UM202	*HfrH thi-1 katG17::Tn10*	21

[a] *E. coli* Genetic Stock Center.

to paraquat and hypochlorous acid resulted in a decrease in intracellular GSH [6,7].

In the present study we compared the effects of two oxidants, H_2O_2 and menadione, on the levels and redox status of intra- and extracellular glutathione in growing *E. coli*. We also measured the activity of three enzymes related to glutathione metabolism, glutathione oxidoreductase (GOR), γ-glutamyltranspeptidase (GGT) and glutathione S-transferase (GST). We found that H_2O_2 and menadione exert distinct effect on these parameters.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and chemicals

Table 1 lists the *E. coli* strains used in this study. All strains were grown overnight at 37°C in M9 medium [11] supplemented with 0.2% glucose, 0.2% Casamino acids, 1 μg thiamine hydrochloride per milliliter, and the appropriate antibiotics. After centrifugation of overnight culture, bacteria were resuspended in the same fresh medium (50 ml in a 250 ml flask) and were cultivated at 37°C with rotary shaking (150 rpm). Growth was monitored by measuring the absorbance at 670 nm. Bacteria were treated with H_2O_2 and menadione in the middle of the log-phase, when culture density was 0.5 g dry cells per liter.

All chemicals for GSH and GSSG measurement, enzyme activity assays, Casamino acids and thiamine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents used in this study were analytical grade.

Determination of GSH and GSSG

Samples of the cell suspensions were removed and centrifuged at 8000 × *g*. The media supernatant was saved for determination of total and oxidized extracellular glutathione. For the determination of intracellular total glutathione, the bacterial pellet was resuspended in 20 mM ethylenediaminetetraacetate and the bacteria were lysed by sonification at 0°C, using a 30 s pulse for six cycles. Perchloric acid (the final concentration 0.5 mM) was added to the lysate to precipitate proteins. The suspension was centrifuged and supernatant was neutralized to pH 7.5 with 5 M KOH, frozen, and centrifuged in order to eliminate the potassium perchlorate. The total glutathione was assayed by the Tietze method [12]. For the determination of the GSSG, N-ethylmaleimide (NEM), (the final concentration 2 mM), was added in order to complex GSH and the bacteria were lysed by sonification. Then, the samples were treated as well as for the determination of intracellular total glutathione. After removal of unreacted NEM by seven extractions with ether the solutions were assayed as above. The concentration of glutathione was calculated by standard plots with the known amounts of GSH and GSSG. The standard samples of GSSG and GSH were treated as well as samples of the cell suspensions.

Determination of enzyme activities

Glutathione reductase activity was determined at 30°C by measuring the rate of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidation at 340 nm with GSSG as the substrate [13]. One unit of enzyme activity reduced 1 μmol of oxidized glutathione per minute.

γ-glutamyltranspeptidase (GGT) activity was measured with L-γ-glutamyl-*p*-nitroanilide as a substrate and glycylglycine as an acceptor [14]. One unit of enzyme activity was defined as the amount of enzyme which transferred 1 μmol of γ-glutamyl moiety per minute.

The activity of glutathione S-transferase (GST) was assayed by the method of Habig et al. [15] with modification of Iizuka et al. [16]. One unit of enzyme activity was defined as the amount producing 1 μmol of conjugate of GSH with 1-chloro-2,4-dinitrobenzene (CDNB) per minute.

Catalase activity in whole cells was assayed by following the disappearance of H_2O_2 at 240 nm [17]. One unit of activity was defined as the amount of enzyme activity catalyzing removal of 1 μmol H_2O_2 per min at 25°C. Protein concentration was determined by the Lowry method with bovine serum albumin as a standard [18].

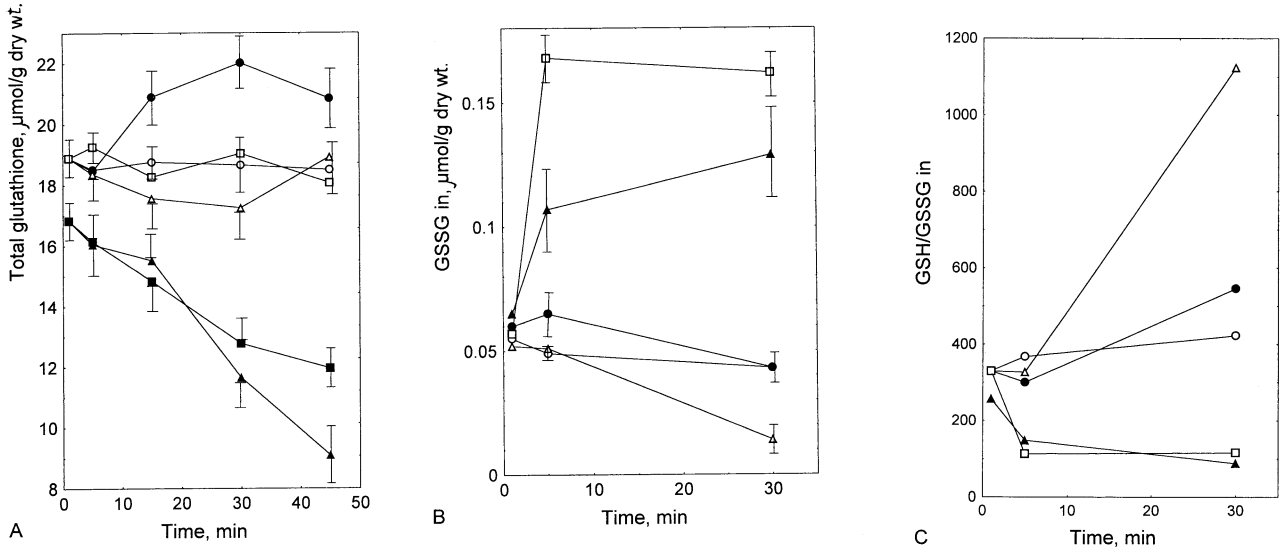

Fig. 1. Effect of H_2O_2 on intracellular levels of (A) total glutathione GSH_{in} + $GSSG_{in}$); (B) GSSG; and (C) the ratio of GSH:GSSG in growing *Escherichia coli*. AB1157 (wt): ○—control; ●—1 mM H_2O_2; △—10 mM H_2O_2; □—25 mM H_2O_2. UM202 (*katG*): ■—control; ▲—10 mM H_2O_2. Error bars show SEMs.

Statistics. Each result is indicated as the mean value of at least three independent experiments ± the standard error of the mean (SEM). Differences were analyzed by Student's *t* test. A *p* value of $< .05$ was used as the minimum for statistical significance.

RESULTS

Effect of H_2O_2 on the levels of intra- and extracellular glutathione in growing E. coli AB1157

The inhibitory effect of H_2O_2 on growth of *E. coli* AB1157 was proportional to the concentration of oxidant. So, treatment of growing cells with 25 mM H_2O_2 caused complete inhibition of growth. When the cells were treated with 10 mM H_2O_2, transient inhibition of growth was observed, and 60 min later growth rate was 25% to that in the control culture. The addition of 1 mM H_2O_2 caused a decrease in growth rate by 33%, after 20 min the growth rate had returned to normal.

In contrast to the effect of H_2O_2 on bacterial growth, any correlation between the concentration of oxidant and intracellular glutathione status was not detected (Fig. 1). Treatment of *E. coli* AB1157 with H_2O_2 concentrations ranging from 1 to 25 mM did not result in an appreciable decrease in intracellular levels of total glutathione (GSH_{in} + $GSSG_{in}$). Moreover, the exposure to 1 mM H_2O_2 resulted in a minor increase in total glutathione (Fig. 1A). During logarithmic growth the ratio of GSH_{in}:$GSSG_{in}$ was varied between 300 and 400. These values are close to those obtained for other wild-types *E. coli* grown into stationary phase [19]. The ratio of GSH_{in}:$GSSG_{in}$ and the level of $GSSG_{in}$ after the addition of 1 mM H_2O_2 were changed insignificantly. Surprisingly, the level of $GSSG_{in}$ reliably decreased and, respectively, the ratio of GSH_{in}:$GSSG_{in}$ increased, when the cells were treated with 10 mM H_2O_2. Both marked increase in $GSSG_{in}$ and a decrease in the ratio of GSH_{in}:$GSSG_{in}$ were observed at 25 mM H_2O_2 (Figs. 1B and 1C).

It has been shown earlier that *E. coli* secretes into the media micromolar amounts of glutathione [20]. Under our conditions growing *E. coli* AB1157 exported approximately 9 μM/g dry cells of total extracellular glutathione. Most of the external glutathione was in the reduced form and, therefore, the ratio of GSH_{out}:$GSSG_{out}$ was sufficiently high (Fig. 2). In contrast to intracellular glutathione, there is certain correlation between H_2O_2 concentration in medium and extracellular glutathione. The exposure to 1 mM H_2O_2 resulted in a moderate decrease in the ratio of GSH_{out}:$GSSG_{out}$ due to a decrease in GSH_{out}. When the cells were treated with 10 mM H_2O_2, transient decrease in extracellular GSH was observed. The measurement of H_2O_2 in medium indicated that an increase in GSH_{out} was started once the most part of oxidant was destroyed and H_2O_2 concentration in medium was below 1.0–1.5 mM (data not shown). Although there was a 10-fold decrease in the ratio of GSH_{out}:$GSSG_{out}$ due to an increase in $GSSG_{out}$, GSH still constituted most part of the total external glutathione (Fig. 2). When the cells were treated with 25 mM H_2O_2, GSH_{out} reduced to a low level. The GSSG became dominant component of total glutathione. Respectively, the ratio of GSH_{out}:$GSSG_{out}$ reduced to 0.4.

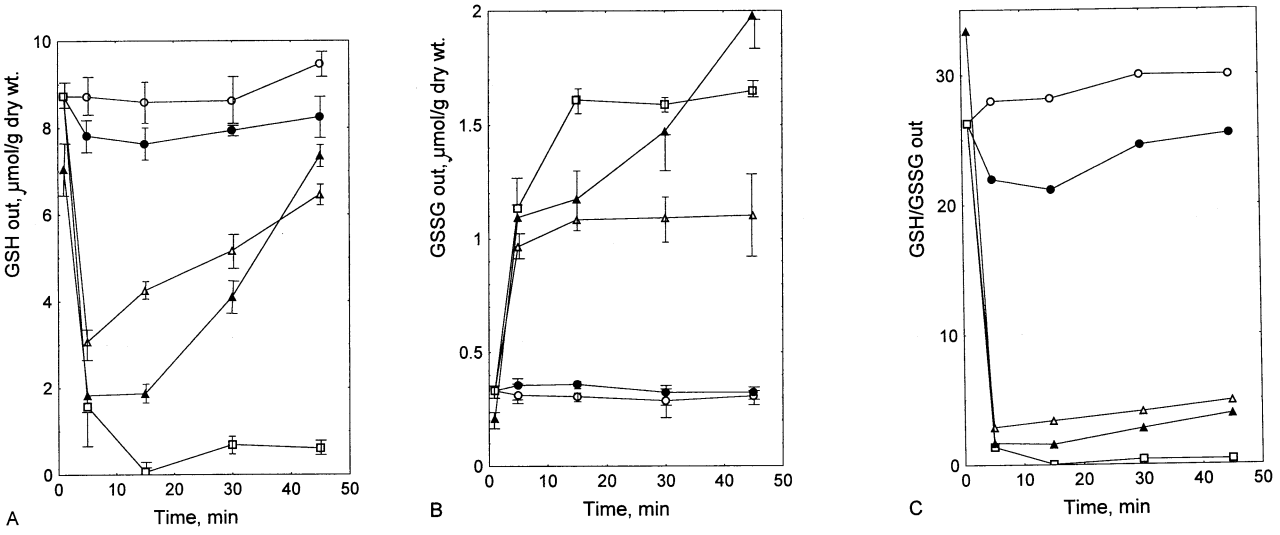

Fig. 2. Effect of H_2O_2 on extracellular levels (A) GSH; (B) GSSG; and (C) the ratio of GSH:GSSG in growing *Escherichia coli*. AB1157 (wt): ○—control; ●—1 mM H_2O_2; △—10 mM H_2O_2; □—25 mM H_2O_2. UM202 (*katG*): ▲—10 mM H_2O_2. Error bars show SEMs.

Taken together, these experiments indicate that there are substantial differences in effect of H_2O_2 on the intra- and extracellular glutathione status.

Effect of H_2O_2 on the levels of intra- and extracellular glutathione in growing E. coli lacking catalase HP-I

E. coli UM202 is a mutant deficient in catalase hydroperoxidase I (HP-I) [21]. In these cells, the basal level of total intracellular glutathione was somewhat below the level in the *katG*$^+$ cells. During aerobic growth the HP-I-deficient cells lost a significant amount of the total intracellular glutathione (Fig. 1A). The exposure to 10 mM H_2O_2 caused a complete growth inhibition. The measurement of H_2O_2 in medium indicated that during the first 15 min after the addition of 10 mM H_2O_2 to growing UM202, the level of oxidant in medium linearly reduced to 7 mM and then remained constant (data not shown). As compared to the parental strain, the catalase-deficient mutant showed the more severe changes in glutathione status in both compartments (Figs. 1B and 1C, and 2B and C). The treatment of UM202 with 10 mM H_2O_2 resulted in a significant increase in concentration of GSSG and a decrease in the GSH:GSSG ratio. In contrast to wild-type *E. coli*, in the HP-I-deficient cells status of extracellular and intracellular glutathione was changed in a similar manner. In summary, these results suggest that in growing *E. coli* catalase HP-I plays an important role in protecting intracellular GSH against oxidative action of H_2O_2.

Effect of menadione on the levels of intra- and extracellular glutathione in growing E. coli AB1157

Exposure of *E. coli* AB1157 to 0.2 mM MD caused a 10% inhibition of growth. A moderate decrease in total intracellular glutathione was accompanied by a 4-fold increase in $GSSG_{in}$ and a decrease in the ratio of GSH_{in}:$GSSG_{in}$ from 370 to 87 (Fig. 3A). One-half millimeter MD caused complete growth inhibition and almost complete depletion of total intracellular glutathione. The level of $GSSG_{in}$ increased 10-fold and the ratio of GSH_{in}:$GSSG_{in}$ decreased to 10 (Figs. 3B and C). The changes in status of extracellular glutathione were similar to those observed for intracellular glutathione. After menadione exposures, a dose-dependent decrease in total extracellular glutathione, an increase in $GSSG_{out}$, and a decrease in the ratio of GSH_{out}:$GSSG_{out}$ was detected (Fig. 4). It should be noted that even in the presence of 0.5 mM MD GSH remained the dominant component of total glutathione in both compartments (Figs. 3 and 4). Remarkably also that unlike menadione, no increase in intracellular and extracellular GSSG was found after exposure to the redox-cycling agent paraquat [6].

Effect of menadione on the levels of intra- and extracellular glutathione in growing E. coli sodAsodB and soxR

E. coli QC909 is a mutant lacking all cytosolic superoxide dismutase activities (*sodAsodB*) [22]. In these cells, the basal level of total intracellular glutathione was somewhat below the level in the sodA^+sodB^+ cells. When cultures were treated with 0.2 mM MD, the SOD-deficient strain showed a more significant decrease in total intracellular glutathione in comparision with its parental strain (Fig. 3). In contrast to wild-type *E. coli*, in *sodAsodB*, a decrease in total intracellular glutathione was not accompanied by an increase in $GSSG_{in}$. As a result in QC909, treated with MD, the ratio of GSH_{in}:

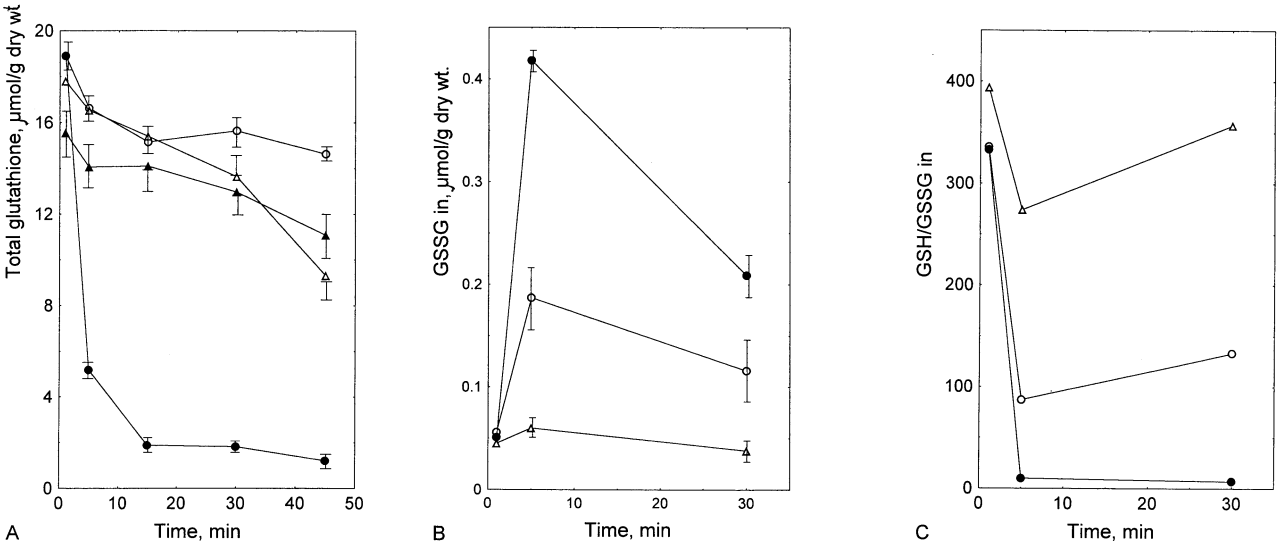

Fig. 3. Effect of menadione on intracellular levels of (A) total glutathione (GSH_{in} + $GSSG_{in}$); (B) GSSG; and (C) the ratio of GSH:GSSG in growing *Escherichia coli*. AB1157 (wt): ○—0.2 mM MD; ●—0.5 mM MD. QC909 (*sodAsodB*): △—0. 2 mM MD. JHC1092 (*soxR*): ▲—0.2 mM MD. Error bars show SEMs.

$GSSG_{in}$ was three times higher than in the wild-type cells (Fig. 3C). In QC909 and its parental strain, the changes in status of extracellular glutathione under the action of MD were similar, but QC909 showed more severe shift toward the oxidized form of glutathione (Fig. 4).

In *E. coli* the *soxR*S regulon coordinates the induction of genes in response to superoxide [23,24]. *E. coli* JHC1092 lacking a functional *soxR* gene are unable to induce the activities controlled by the *soxR*S regulon including sodA, the product of which is Mn-containing superoxide dismutase. The changes in glutathione status in these cells were close to that of SOD-deficient strain (Figs. 3 and 4).

Effect of oxidants on enzymatic activities

Exposure of *E. coli* AB1157 to 1 mM H_2O_2 or 0.2–0.5 mM MD resulted in only a small increase in specific activity of GOR. No changes in GOR activity were found after exposure to 10 mM H_2O_2 (Table 2). Unlike H_2O_2, MD produced a dose-dependent inhibition of glutathione S-transferase activity. A statistically significant increase

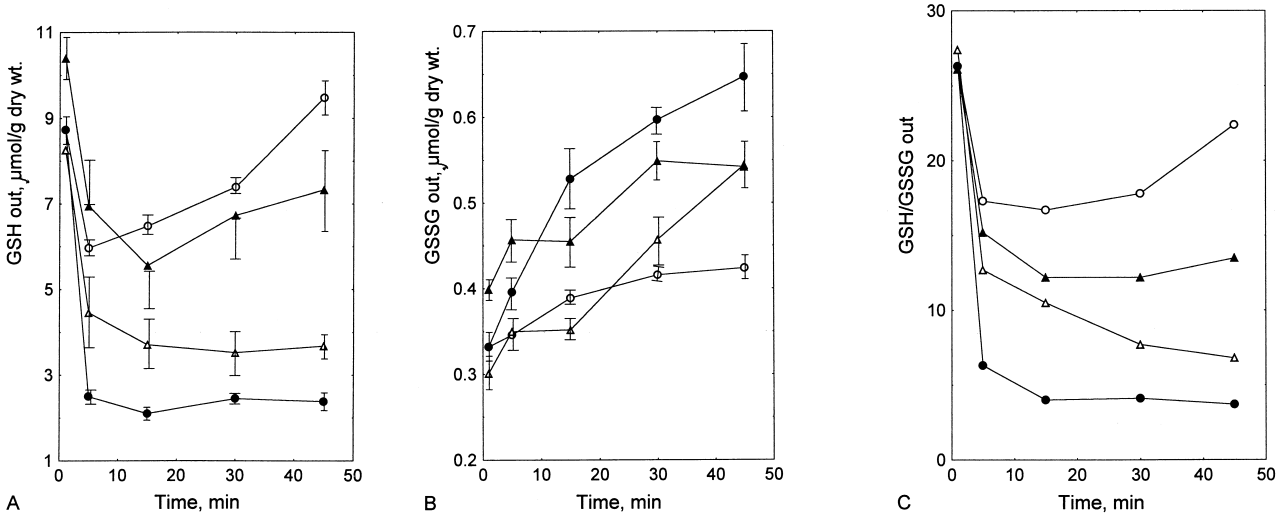

Fig. 4. Effect of menadione on extracellular levels of (A) GSH; (B) GSSG; and (C) the ratio of GSH:GSSG in growing *Escherichia coli*. AB1157 (wt): ○—0.2 mM MD; ●—0.5 mM MD. QC909 (*sodAsodB*): △—0.2 mM MD. JHC1092 (*soxR*): ▲—0.2 mM MD. Error bars show SEMs.

Table 2. Effect of Oxidants on Enzymatic Activities in *E. coli* AB1157

Treatment	Enzyme activities			
	GOR[a]	GST[a]	GGT[a]	Catalase[b]
Control	67 ± 5 (1.0)	368 ± 42 (1.0)	1.7 ± 0.1 (1.0[c] 1.3 ± 0.1 (1.0)[d]	2.4 ± 0.3 (1.0)
1 mM H_2O_2	86 ± 6 (1.3)*	347 ± 67 (0.9)	2.1 ± 0.3 (1.2) 1.6 ± 0.1 (1.2)*	24.6 ± 1.0 (10.)†
10 mM H_2O_2	71 ± 6 (1.1)	391 ± 34 (1.1)	2.1 ± 0.1 (1.2)† 1.7 ± 0.1 (1.3)*	5.4 ± 0.6 (2.2)*
Control	64 ± 5 (1.0)	339 ± 26 (1.0)	1.7 ± 0.1 (1.0) 1.3 ± 0.1 (1.0)	1.8 ± 0.2 (1.0)
0.2 mM MD	103 ± 6 (1.6)†	225 ± 29 (0.75)	ND	46 ± 10 (25)†
0.5 mM MD	87 ± 6 (1.4)*	129 ± 20 (0.4)†	1.8 ± 0.2 (1.06) 1.3 ± 0.2 (1.0)	49 ± 8 (27)†

[a] Milliunits per milligram of protein.
[b] Milliunits per milligram of protein.
[c] Transferase activity.
[d] Hydrolase activity.
* $p < .05$ and † $p < .01$, respectively, compared with control.
ND = not determined.

in γ-glutamyltranspeptidase (GGT) activity was observed only after exposure to 10 mM H_2O_2. Although there were little or no changes in the activity of enzymes related to glutathione metabolism, the catalase activity increased several times after exposure to both oxidants (Table 2).

DISCUSSION

The results presented here show that the effects of MD and H_2O_2 on the intracellular glutathione status in wild-type *E. coli* are different. Whereas MD caused a drastic decrease in total glutathione and the ratio of GSH_{in}:$GSSG_{in}$, H_2O_2 did not result in an appreciable decrease in total glutathione even after the high oxidant exposures, and the exposure to 1–10 mM resulted in an increase in the ratio of GSH_{in}:$GSSG_{in}$. Behavior of *katG* and *sodAsodB* mutants provides a possible explanation of the obtained results. In *katG* mutant exposed to H_2O_2 the level of total intracellular glutathione decreased, $GSSG_{in}$ increased and the ratio of GSH_{in}:$GSSG_{in}$ reduced to low levels. It seems likely that in the absence of catalase HP-I, H_2O_2 freely penetrates into cells and promotes oxidation of GSH and a decrease in total glutathione. Thus, in growing wild-type *E. coli* the periplasmic catalase HP-I [21,25] has high protective effect on oxidation of the intracellular GSH by exogenous H_2O_2.

In contrast to wild-type cells, treatment of *sodAsodB* with MD did not lead to an increase in intracellular GSSG. A decrease in the ratio of GSH_{in}:$GSSG_{in}$ in *sodAsodB* was less pronounced than in the wild-type cells, and it was due to a decrease in GSH and not to an increase in GSSG. What are possible reasons for reduced GSSG production in *sodAsodB* cells? There is certain evidence in favor of the participation of H_2O_2 in oxidation of intracellular GSH after treatment of wild type *E. coli* with MD. It was reported that in vitro the interaction of MD with GSH led to GSSG formation and this reaction was inhibited by catalase while superoxide dismutase had a little effect. It was concluded that H_2O_2 is an important determinant of GSH oxidation in this system [26].

In vivo H_2O_2 is produced within aerobic cells during metabolism of the redox-cycling drugs by spontaneous and superoxide dismutases (SODs)-catalyzed dismutation of superoxide anion [27]. The lack of SOD activity in *E. coli sodAsodB*, treated with MD must decrease production of intracellular H_2O_2. If in cells treated with menadione H_2O_2 contributes sigificantly to the GSH oxidation, it may be expected that GSSG levels in *sodAsodB* cells will be somewhat below that in wild type. The data in Fig. 3B show that this was the case. In summary, a possible reason for the different effect of H_2O_2 and MD on intracellular glutathione status in wild type is oxidation of GSH by intracellular H_2O_2, produced during the metabolism of MD. It seems likely that in contrast to exogenous H_2O_2, catalase HP-I has lesser protective effect on oxidation of the intracellular GSH by endogenous H_2O_2.

An increase in extracellular GSSG after MD and high doses H_2O_2 was detected in all strains investigated. It is known that eukariotic cells treated with oxidants export GSSG into the medium [3]. Because simultaneously extracellular GSH decreased (Fig. 3), it is an open ques-

tion whether an increase in extracellular GSSG is due to the export of intracellular GSSG or oxidation of extracellular GSH.

GOR catalyzes the NADPH dependent reduction of GSSG to GSH. It was reported that in *E. coli* GOR is inhibited by paraquat [28]. The present study showed that in wild-type *E. coli* treated with MD or H_2O_2 the GOR activity increased slightly. These data favor the view that in *E. coli* GOR plays a minor role in the cell response to H_2O_2 stress [29].

The interaction of MD with GSH leads also to a formation of menadione-GSH conjugate [26,30]. A formation of GSH conjugates with electrofiles is catalyzed by glutathione S-transferases (GSTs) [1,3]. The enzyme possessing properties of GST was purified from *E. coli* [16]. This study showed that GST activity was inhibited by MD. It is possible that MD inhibits GST activity by the indirect route. It was reported that O_2^-, which is produced during the metabolism of the redox-cycling drugs, excises iron from [4Fe-4S] clusters of several dehydratases [28] and increases the level of free iron into the cytosol [31]. It was shown that in vitro the activity of GST from *E. coli* was markedly inhibited by Fe^{2+} [16].

In these experiments, the responses of *E. coli* to oxidative stress induced by menadione or the *katG* mutation were accompanied by a decrease in total glutathione. One possible way to decrease intracellular glutathione is its degradation. In eukariotic cells enzyme γ-glutamyltranspeptidase (GGT) catalyzes the first step in the catabolism of glutathione and the transmembrane translocation of glutathione [1]. Previous studies showed that GGT was important also for oxidant-challenged cells and menadione increased GGT activity in rat cells [32]. The physiological roles of the enzyme in bacterial cells are not entirely known. In this study a small increase in GGT activity was observed only after exposure to 10 mM H_2O_2. A question about the role of GGT in response of *E. coli* to oxidative stress remains open.

What role may the changes in the redox state of intracellular glutathione on the response of *E. coli* to oxidative stress play? It is known that one of the roles of GSH is related to its participation in the regulation of cellular metabolism by changes in redox state of protein thiols. The ratio GSH:GSSG is an important parameter in this process [2]. In *E. coli*, two proteins OxyR and SoxR govern genetic responses to oxidative stress and have key redox-active sites formed by cysteine residues. It was recently reported that OxyR is activated through the formation of a disulfide bond and is deactivated by enzymatic reduction with glutaredoxin 1 [33]. SoxR contains reduced iron-sulfur clusters and it is activated upon oxidation of these clusters [34,35]. It was reported that a decrease in intracellular GSH exerts a positive effect on the transcription activity of OxyR and SoxR. In vivo the induction by paraquat of SoxR-dependent transcription was much higher in a GSH-deficient *E. coli* strain than in GSH^+ parent [36]. We previously showed that pretreatment of growing *E. coli* with ferricyanide decreased intracellular glutathione and accelerated the H_2O_2-induced expression of the *katG* gene controlled by OxyR. The H_2O_2-inducible catalase activity was markedly higher in GSH-deficient cells than in GSH^+ strain [37]. Considering these data, it may be expected that the changes in redox status of intracellular glutathione observed after H_2O_2 or MD treatments will exert influence on the induction of genes controlled by oxyR and *soxR*S regulons.

In addition to the above data, other data can testify in favor of this assumption. Evidence was obtained that the *soxR*S regulon can respond to a decrease in the NADPH:nicotinamide adenine dinucleotide phosphate $(NADP)^+$ ratio [38]. Two redox couples NADPH:$NADP^+$ and GSH:GSSG contribute to redox potential of cytoplasm and their metabolism have some common points. At present, no evidence was found for the direct participation of glutathione in defense against MD and H_2O_2 in growing *E. coli*. The role of glutathione on the cell response to oxidative stress can be realized by the indirect route through influence of the glutathione redox status on total redox potential of cytoplasm.

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REFERENCES

[1] Meister, A.; Anderson, M. E. Glutathione. *Ann. Rev. Biochem.* **52:**711–760; 1983.
[2] Brigelius, R. Mixed disulfides: biological functions and increase in oxidative stress. In: Sies, H., ed. *Oxidative stress.* London: Academic Press; 1985:243–272.
[3] Kosower, N. S.; Kosower, E. M. The glutathione status of cells. *Int. Rev. Cytol.* **54:**109–160; 1978.
[4] Bellomo, G.; Thor, H.; Eklöw-Låstbom, Nicotera, P.; Orrenius, S. Oxidative stress–mechanisms of cytotoxicity. *Chem. Scripta* **27A:** 117–120; 1987.
[5] Greenberg, J. T.; Demple, B. Glutathione in *Escherichia coli* is dispensable for resistance to H_2O_2 and gamma radiation. *J. Bacteriol.* **168:**1026–1029; 1986.
[6] Romero, M. J.; Canada, A. T. The evaluation of *Escherichia coli* as a model for oxidant stress in mammalian hepatocytes: role of glutathione. *Toxicol. Appl. Pharmacol.* **111:**485–495; 1991.
[7] Chesney, J. A.; Eaton, J. W.; Mahoney, J. R., Jr. Bacterial glutathione: a sacrificial defense against chlorine compounds. *J. Bacteriol.* **178:**2131–2135; 1996.
[8] Fahey, R. C.; Brown, W. C.; Adams, W. B.; Worsham, M. B. Occurrence of glutathione in bacteria. *J. Bacteriol.* **33:**1126–1129; 1978.
[9] Smirnova, G. V.; Oktyabrsky, O. N. Near-ultraviolet radiation and hydrogen peroxide modulate intracellular levels of potassium and thiols in *Escherichia coli*. *Curr. Microbiol.* **28:**77–79; 1994.
[10] Greenberg, J. T.; Chou, J. H.; Monach, P. A.; Demple, B. Activation of oxidative stress genes by mutations at the soxQ/cfxB/

marA locus of *Escherichia coli*. *J. Bacteriol.* **173:**4433–4439; 1991.
[11] Miller, J. H.*Experiments in molecular genetics.* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1972.
[12] Tietze, F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Anal. Biochem.* **27:**502–522; 1969.
[13] Scrutton, N. S.; Berry, A.; Perham, R. N. Purification and characterization of glutathione reductase encoded by a cloned and over-expressed gene in *Escherichia coli*. *Biochem. J.* **245:**875–880; 1987.
[14] Suzuki, H.; Kumagai, H.; Tochikura, T. γ-glutamyltranspeptidase from *Escherichia coli* K-12: Purification and properties. *J. Bacteriol.* **168:**1325–1331; 1986.
[15] Habig, W. H.; Pabst, M. J.; Jakoby, W. B. Glutathione S-transferase: first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* **249:**7130–7139; 1974.
[16] Iizuka, M.; Inoue, Y.; Murata, K.; Kimura, A. Purification and some properties of glutathione S-transferase from *Escherichia coli* B. *J. Bacteriol.* **171:**6039–6042; 1989.
[17] Beers, R. F.; Sizer, I. W. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.* **196:**133–140; 1952.
[18] Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:**265–275; 1951.
[19] Alonso-Moraga, A.; Bocanegra, A.; Torres, J. M.; López-Barea, J.; Pueyo, C. Glutathione status and sensitivity to GSH-reacting compounds of *Escherichia coli* strains deficient in glutathione metabolism and/or catalase activity. *Mol. Cell. Biochem.* **73:**61–68; 1987.
[20] Owens, R. A.; Hartman P. E. Export of glutathione by some widely used *Salmonella typhimurium and Escherichia coli* strains. *J. Bacteriol.* **168:**109–114; 1986.
[21] Triggs-Raine, B. L.; Loewen, P. C. Physical characterization of *katG*, encoding catalase HPI of *Escherichia coli*. *Gene* **52:**121–128; 1987.
[22] Carlioz, A.; Touati, D. Isolation of superoxide dismutase mutants in *Escherichia coli*: is superoxide dismutase necessary for aerobic life? *EMBO J.* **5:**623–630; 1986.
[23] Greenberg, J. T.; Monach, P.; Chou, J. H.; Josephy, P. D.; Demple B. Positive control of a global antioxidant defense regulon activated by superoxide-generating agents in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **87:**6181–6185; 1990.
[24] Tsaneva, I. R.; Weiss, B.*soxR*, a locus governing a superoxide response regulon in *Escherichia coli* K-12. *J. Bacteriol.* **172:** 4197–4205; 1990.
[25] Heimberger, A.; Eisenstark, A. Compartmentalization of catalases in *Escherichia coli*. *Biochem. Biophys. Res. Commun.* **154:**392–397; 1988.
[26] Ross, D.; Thor, H.; Orrenius, S.; Moldeus, P. Interaction of menadione (2-methyl-1,4-naphthoquinone) with glutathione. *Chem. Biol. Interactions* **55:**177–184; 1985.
[27] Fridovich, I. Superoxide radical: An endogenous toxicant. *Annu. Rev. Pharmacol. Toxicol.* **23:**239–257; 1983.
[28] Liochev, S. I.; Fridovich, I. Paraquat diaphorases in *Escherichia coli*. *Free Radic. Biol. Med.* **16:**555–559; 1994.
[29] Becker-Hapak, M.; Eisenstark, A. Regulation of glutathione oxidoreductase by *rpoS* in *Escherichia coli*. *FEMS Microbiol. Lett.* **134:**39–44; 1995.
[30] DiMonte, D.; Ross, D.; Bellomo, G.; Eklöw, L.; Orrenius, S. Alterations in intracellular thiol homeostasis during the metabolism of menadione by isolated rat hepatocytes. *Arch. Biochem. Biophys.* **235:**334–342; 1984.
[31] Keyer, K.; Imlay, J. A. Superoxide accelerates DNA damage by elevating free- iron levels. *Proc. Natl. Acad. Sci USA* **93:**13635–13640; 1996.
[32] Kugelman, A.; Choy, H. A.; Liu, R.; Shi, M. M.; Gozal, E.; Forman, H. J. γ-glutamyl transpeptidase is increased by oxidative stress in rat alveolar L2 epithelial cells. *Am. J. Respir. Cell. Mol. Biol.* **11:**586–592; 1994.
[33] Zheng, M.; Aslund, F.; Storz, G. Activation of the OxyR transcription factor by reversible disulfide bond formation. *Science* **279:**1718–1721; 1998.
[34] Gaudu, P.; Weiss, B. SoxR, a [2Fe-2S] transcription factor, is active only in its oxidized form. *Proc. Natl. Acad. Sci. USA* **93:**10094–10098; 1996.
[35] Ding, H.; Hidalgo, E.; Demple, B. The redox state of the [2Fe-2S] clusters in SoxR protein regulates its activity as a transcription factor. *J. Biol. Chem.* **271:**33173–33175; 1996.
[36] Ding, H.; Demple, B. Glutathione-mediated destabilization in vitro of [2Fe-2S] centers in the SoxR regulatory protein. *Proc. Natl. Acad. Sci. USA* **93:**9449–9453; 1996.
[37] Smirnova, G. V.; Muzyka, N. G.; Glukhovchenko M. N.; Oktyabrsky, O. N. Effects of penetrating and non-penetrating oxidants on *Escherichia coli*. *Biochemistry* **62:**480–484; 1997.
[38] Liochev, S. I.; Fridovich, I. Fumarase C, the stable fumarase of *Escherichia coli*, is controlled by the *soxR*S regulon. *Proc. Natl. Acad. Sci. USA* **89:**5892–5896; 1992.

ABBREVIATIONS

soxR and *oxyR*—in italic (*soxR* and *oxyR*) when designated as a gene, OxyR and SoxR when designated as protein product.
CDNB—1-chloro-2,4-dinitrobenzene
EDTA—ethylenediaminetetraacetic acid
GGT—γ-glutamyltranspeptidase
GOR—glutathione oxidoreductase
GSH_{in} and GSH_{out}—intra- or extracellular reduced glutathione
GST—glutathione S-transferase
HPI—catalase-hydroperoxidase I
MD—menadione
NADPH—nicotinamide adenine dinucleotide phosphate (reduced)
NADP—nicotinamide adenine dinucleotide phosphate (oxidizd)
NEM—N-ethylmaleimide
PQ—paraquat
SOD—superoxide dismutase