Tellurite-mediated thiol oxidation in
Escherichia coli

Raymond J. Turner, Joel H. Weiner and Diane E. Taylor

The oxyanion of tellurium, tellurite (TeO\(_{2}^{–}\)), is toxic to most micro-organisms, particularly Gram-negative bacteria. The mechanism of tellurite toxicity is presently unknown. Many heavy metals and oxyanions, including tellurite, interact with reduced thiols (RSH). To determine if tellurite interaction with RSH groups is involved in the toxicity mechanism, the RSH content of Escherichia coli cultures was assayed. After exposure to tellurite, cells were harvested and lysed in the presence of the RSH-specific reagent 5,5'-dithiobis(2-nitrobenzoic acid). Upon exposure of tellurite-susceptible cells to TeO\(_{2}^{2–}\), the RSH content decreased markedly. Resistance to potassium tellurite (Te\(^{0}\)) in Gram-negative bacteria is encoded by plasmids of incompatibility groups IncFI, IncP\(_{2}\), IncHI2, IncHI3 and IncHI1, as well as the tehA-tehB operon from the E. coli chromosome. When cells harbouring a Te\(^{0}\) determinant were exposed to TeO\(_{2}^{2–}\), only a small fraction of the RSH content became oxidized. In addition to tellurite-dependent thiol oxidation, the resistance of E. coli mutants affected in proteins involved in disulfide-bond formation (dsb) was investigated. Mutant strains of dsbA and dsbB were found to be hypersensitive to tellurite (MIC 0.008–0.015 µg K\(_{2}\)TeO\(_{3}\) ml\(^{–1}\) compared to wild-type E. coli with MICs of 1–2 µg K\(_{2}\)TeO\(_{3}\) ml\(^{–1}\)). In contrast, dsbC and dsbD mutants showed no hypersensitivity. The results suggest that hypersensitivity to tellurite is reliant on the presence of an isomerase activity and not the thiol oxidase activity of the Dsb proteins. The results establish that the Te\(^{0}\) determinants play an important role in maintaining homeostasis of the intracellular reducing environment within Gram-negative cells through specific reactions with either TeO\(_{2}^{2–}\) or thiol:tellurium products.

Keywords: tellurite, resistance, thiol oxidation, heavy metal toxicity, Dsb

INTRODUCTION

The oxyanion of tellurium, tellurite (TeO\(_{2}^{2–}\)), is toxic to most micro-organisms, particularly Gram-negative bacteria. Bacteria turn black upon exposure to tellurite as a result of the deposition of elemental tellurium (Te\(^{0}\)) within the cell (Lloyd-Jones et al., 1994; Taylor et al., 1988). This phenomenon has also been reported in yeast (Liangyau et al., 1993) and erythrocytes (Deuticke et al., 1992). It has long been considered that the toxicity of tellurite is due to general oxidation (Summers & Jacoby, 1977). However, there is little foundation for this assumption and given a standard reduction potential of -1.186 V for the reaction TeO\(_{2}^{2–}\) + 3H\(_{2}\)O + 4e\(^{–}\) → Te\(^{0}\) + 6OH\(^{–}\), it appears unlikely that tellurite is a random oxidant, and specific tellurite reduction biochemistry probably occurs. NAD(P)H-dependent tellurite reductase activity has been observed in Mycobacterium avium (Terai et al., 1958), Thermus thermophilus HB8 (Chiong et al., 1988) and Bacillus stearothermophilus V (Moscoso et al., 1998). However, no such activity has been observed in Escherichia coli (R. J. Turner, unpublished results). One key site of tellurite reduction has been identified to be the membrane-associated NarG and NarZ nitrate reductases (Avazeri et al., 1997), which specifically catalyse the reduction of TeO\(_{2}^{2–}\). This activity significantly contributes to the basal level of resistance found in E. coli. Preliminary evidence suggests that the cytochrome \(d\) terminal oxidase also has tellurite reductase activity (Trutko et al., 1998).

Abbreviations: DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); Te\(^{0}\), tellurite resistant; RSH, reduced thiol(s).
Resistance to potassium tellurite has been found in various Gram-negative bacteria and is usually plasmid encoded. At least five different tellurite-resistance (Te') determinants have been identified through genetic studies and DNA sequencing (see review by Taylor, 1999); such determinants are both chromosomal and plasmid encoded. The plasmid incompatibility groups in which tellurite resistance has been identified include: IncHII and IncHIII (Bradley et al., 1982; Summers & Jacoby, 1977; Taylor & Summers, 1979), IncHIII (Whelan, 1992), IncPα (Bradley, 1985) and IncF (Turner et al., 1992). In addition, there are a number of unclassified plasmids which confer Te' on their hosts (Taylor & Summers, 1979). Te' determinants found on chromosomes include the tehAtehB operon at 32-3 min of the E. coli chromosome (Taylor et al., 1994), tmp of Pseudomonas syringae (Cournoyer et al., 1998) and the trgAB genes of Rhodobacter sphaeroides (O’Gara et al., 1997).

The majority of the tellurite-resistance research to date has centred on the ter operon from the IncH plasmids (Hill et al., 1993; Jobling & Ritchie, 1987, 1988; Taylor et al., 1994; Whelan, 1992; Whelan et al., 1995, 1997) and the kilA (or kla) operon from the IncPα plasmid RK2 (Bradley, 1985; Taylor & Bradley, 1987; Thomas et al., 1980; Turner et al., 1994a, b, 1995b; Walter & Taylor, 1989). Research has also focused on the teh genes from the E. coli chromosome (Taylor et al., 1994; Turner et al., 1995a, b, 1997; Walter et al., 1991b). Despite considerable effort, the biochemical mechanism of tellurite resistance, either plasmid mediated or chromosomally encoded, is still poorly understood. However, considerable insights have arisen in the understanding of tellurite biochemistry in E. coli. A study examining the resistance levels in metabolic mutants suggests that thiol redox enzymes (glutathione reductase and thioredoxin reductase) and their metabolites (thioredoxin, glutaredoxin and glutathione) are involved in tellurite resistance (Turner et al., 1995b). Superoxide dismutase was also shown to be required. It is clear from that study that resistance mediated by the Te' determinant for the kilA locus and teh have different biochemical requirements (Turner et al., 1995b). The teh locus has a strict requirement for electron transport processes and/or thiol redox coupling whereas the kilA determinant showed no such dependency.

The goal of the present study was to explore the mechanism of tellurite toxicity through examination of the oxyanion’s effects on cell thiols. The effect on RSH upon the exposure of E. coli cells to TeO₃⁻ was examined and the influence of Te' determinants on this effect considered. To further examine the effects of tellurite on thiol biochemistry, proteins involved in disulfide-bond formation (Dsb) within the periplasm were examined. Although cysteines in proteins residing in the cytoplasm are typically in the reduced form, cysteines become oxidized in the periplasm. Dsb proteins are involved in mediating disulfide-bond formation and contribute to proper protein folding (Bardwell et al., 1991). We have examined the tellurite sensitivity in a number of dsb mutants, as well as the resistance levels of Te' determinants within such mutants.

METHODS

Bacterial strains, plasmids, and growth conditions. Te' determinants used in this study are described in Table 1. Host E. coli strains used in this study were: HB101 (Boyer & Roulland-Dussioy, 1969), J53-1 (Bachmann, 1972), MV10 (Thomas et al., 1980) and AB1157 (Bachmann, 1972). Disulfide-bond-formation mutants (dsb) and plasmids (Table 2) were provided by A. Rietsch in J. Beckwith’s group (Department of Microbiology and Molecular Genetics, Harvard Medical School, USA). The strains obtained from this source include R189 [araD139 ara714 (lac)X74 galU galK rpsL thi phoR], R190 (R189; dsbA::kan), R317 (R189; dsbB::kan), R121[R189; ara: leu dsbA::kan], JCB819 (R189; zig12.1::Tn10 malf flaC Z702 dsbB::kan) (Jander et al., 1994), R1179 (R189; dsbC::Cam), R1242 (R189; dsbD::TnCam), R1318 (R189; dsbB::kan dsbD::TnCam), R1361 (R189; dsbA::kan dsbD::TnCam), R1385 (R189; dsbB::kan dsbC::Cam dsbD::TnCam cadC::Tn10).

Additionally, similar strains were obtained from Koreaki Ito (Department of Cell Biology, Kyoto University, Japan). These included CU141 as a K-12 wild-type strain, SS140 (CU141; dsbA33::Tn5), SS141 (CU141; dsbB::kan5) (Kishigami et al., 1995).

All bacterial cultures were grown overnight in Luria–Bertani (LB) broth medium containing the appropriate antibiotics for plasmid maintenance. Antibiotics were used at the following concentrations (µg ml⁻¹): ampicillin, 100; chloramphenicol, 42.5; kanamycin sulfate, 40; tetracycline, 12.5; streptomycin, 50.

The tac promoter in the plasmids pMS1558 and pMStehAB is under expression control by the lacIq gene located on the plasmid which can be induced with IPTG.

MICs. The procedure was performed as previously described (Turner et al., 1995b). Briefly, overnight cultures grown in LB broth were diluted to 10⁶ cells ml⁻¹ and 10 µl aliquots were spotted onto LB agar plates containing serial dilutions of potassium tellurite (Sigma). The MICs were repeated at least three times with identical results.

Reduced thiol (RSH) assay. Overnight cultures were used as a source of a 1% inoculum into a 50 ml flask containing 10 ml LB. The culture was incubated at 37 °C in a water-filled shaker bath for 4 h. For strains in which the Te' determinant required induction (strains harbouring pMS1558 and pMStehAB), the culture was incubated at 37 °C for 2 h and then IPTG was added to a concentration of 1 mM. The culture was incubated for a further 2 h. Cultures were then placed at room temperature (21 °C) and allowed to equilibrate at this temperature for 15 min. To initiate the experiment, K₂TeO₃ was added from a stock solution (10 mg ml⁻¹) to give a final concentration of 50 µg ml⁻¹ (197 µmol l⁻¹). This concentration of tellurite is well below the MIC of the Te' determinants (MICs 64-1024 µg ml⁻¹) and well above the intrinsic resistance levels of the E. coli strains used here (MIC 1-2 µg ml⁻¹) (Table 1). Samples of 500 µl were removed into microfuge tubes at various time intervals and centrifuged at 15000 g for 1 min to pellet the cells. The cell pellets were then frozen and stored at −20 °C until the termination of the experiment. The frozen pellets were thawed and resuspended by vortexing in 1 ml of a freshly prepared solution of 50 mM Tris/HCl pH 8.0, 5 mM
EDTA, 0.1% SDS and 0.1 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB). These cell suspensions were incubated at 37 °C for 30 min, briefly vortexed and then centrifuged in a microfuge for 10 min at 15,000 g. A split-beam spectrometer was utilized, allowing for the sample to be zeroed against the reagent buffer which had been processed as above. The absorbance of the supernatant of each fraction was read at 412 nm. The absorption coefficient of oxidized DTNB (1% SDS and 0.1 M EDTA, 0.1 mM DTNB) at this wavelength was then used to calculate the RSH content of the cell. Additional samples were removed at t = 0 and 120 min for the determination of cell protein concentration (Markwell et al., 1978). This allowed for the RSH content to be normalized to total cell protein. The concentration of RSH in µmol (g cell protein)−1 at t = 0 min was then subtracted from a given samples data set. Thus the data presented are the amount of RSH oxidized as a result of toxin exposure observed over a 2 h time frame. All experiments were performed several times and the data reported are means of at least three independent experiments. The means and standard deviations were calculated and are reported in Figs 1–4.

RESULTS

Tellurite-mediated thiol oxidation in *E. coli*

Exposure of *E. coli* cultures to K$_2$TeO$_3$ (50 µg ml$^{-1}$) resulted in a loss of cellular RSH content (Fig. 1). The RSH concentration was measured by Ellman’s reagent (DTNB), an effective reagent to quantify RSH in peptides and proteins (Means & Feeney, 1971; Lundblad, 1995). The RSH content of the cells was measured in the presence of EDTA and SDS in order to generate cell lysis and protein-denaturing conditions...
which would expose all cysteines to the reagent. The loss of RSH content occurred concurrently with the loss of culture viability and was similar for all wild-type strains of E. coli studied. No difference was observed in the thiol oxidation response curve between the host strain HB101 and HB101 harbouring a control plasmid (pUC8). The data shown in Fig. 1 are the mean of seven independent trials. Thiol oxidation, as observed as a decrease in the amount of RSH, began immediately after addition of tellurite to the culture and continued until the amount of thiol oxidation stabilized, which occurred after about 2 h with incubation at room temperature (21 °C). The rate of thiol oxidation was greater, and stabilized earlier, with higher concentrations of K₂TeO₃ or at higher temperatures (results not shown). In a typical experiment, TeO₃⁻ was in 5–10 molar excess over RSH. The stabilization is likely due to the target thiol oxidation levels as that of azide and cyanide.

Effect of other antimicrobial agents on thiol oxidation

To determine if the oxidation of thiols (the loss of RSH content) is tellurite-dependent or a result of cell death, alternative toxic compounds were examined. Fig. 2 shows the effects of azide, cyanide and paraquat on RSH content. Although the RSH content decreased by approximately 20 μmol RSH (g protein)⁻¹ after 2 h exposure, the level of thiol oxidation is clearly not as extensive as that caused by exposure to tellurite. The greatest loss of thiols caused by toxin exposure was due to paraquat. The extra thiol oxidation from paraquat exposure is likely due to oxidation via the superoxide radical that is produced by this compound. Exposure to antibiotics such as rifampicin was also investigated, which showed thiol oxidation levels similar to azide and cyanide (results not shown). We can conclude from these control data that the majority of the thiol oxidation (loss of RSH content) observed with TeO₃⁻ is directly due to tellurite exposure.

The effects of other metals (Hg²⁺, Cd²⁺, Co²⁺, Cu²⁺, Zn²⁺) and oxyanions of metals and metalloids (AsO₃⁻, AsO₄⁻, CrO₄⁻, SeO₃⁻, SeO₄⁻, TeO₄⁻) on thiol oxidation within whole cells were also examined. Cultures were exposed to the metal toxin at a concentration of 10 mM, in the same manner as for tellurite. Only mercury exposure gave rise to a higher rate of RSH reactivity than tellurite (Hg²⁺ decay shown in Fig. 2). Tellurate (TeO₃⁻) exposure showed the next greatest level, with typically 20% less thiol oxidation than tellurite. All other metal compounds demonstrated similar thiol oxidation levels as that of azide and cyanide.

Effect of Te⁺ determinants on thiol oxidation

Thiol oxidation (loss of RSH content) resulting from tellurite exposure is significantly decreased in the presence of the Te⁺ determinants teh, ter and kilA. Representative thiol oxidation curves for E. coli HB101 containing different Te⁺ determinants is shown in Fig. 3. It is clear from these results that in the presence of the Te⁺ plasmids the total RSH content in the cell is protected from the tellurite-mediated oxidation processes. Levels of thiol oxidation are compared in Fig. 4 for different plasmids and Te⁺ subclones from each of the different Te⁺ determinants.

The cloned operon tehA-tehB from the E. coli chromosome gives resistance to tellurite upon overexpression. Examination of RSH content in cultures harbouring

![Fig. 2. Loss of RSH content in HB101 as a result of exposure to various antibacterial agents: none (control) (△), 10 mM azide (○), 10 mM cyanide (○), 10 mM paraquat (○) and 10 mM HgCl₂ (□).](image)

![Fig. 3. Effect of exposure to K₂TeO₃(50 μg ml⁻¹) on E. coli HB101 harbouring Te⁺ plasmids: (a) pUC8 (control) (□), pTW100 (○) and pRK2Te⁺ (○); (b) pMURTLE (□), pMJ606 (○), pMRP233 (○) and pH510a (△).](image)
plasmid pTWT100, which utilizes the wild-type promoter, demonstrated sustainable protection to thiol oxidation (Fig. 3a). Further overexpression of tehAtelB by expressing the operon behind a tac promoter (pMStehAB) gave rise to even greater levels of RSH protection (Fig. 4). E. coli strains which were deleted in the teh operon did not display increased levels of tellurite-dependent thiol oxidation (data not shown), nor were they hypersensitive to tellurite (Taylor et al., 1994).

A similar level of RSH protection is observed with the Te\textsuperscript{e} determinant from the kilA locus on the IncP\textalpha plasmid RK2\textsuperscript{TelR}. Overexpression of klaAklaBtelB [previously referred to as kilAtelAB (Walter et al., 1991a), where telB is the resistant derivative of klaB] behind the tac promoter gave rise to a greater level of RSH protection, similar to the effect that was seen with overexpression of the teh determinant. This suggests that increased thiol protection correlates with gene expression.

Cultures harbouring the ter Te\textsuperscript{e} determinants from the plasmid incompatibility groups IncHII, IncHI2 and IncHI3 were protected from the tellurite-dependent thiol oxidation (Figs 3b, 4). The variability between different parent plasmids and subclones within the IncH plasmids may result from each of the plasmids and subclones harbouring different constructs of the complete ter gene cluster (terWXYZABCDEF). We do not yet fully understand the interaction among all the genes within this cluster, nor do we know the roles they play in the phenotypes associated with them (Whelan et al., 1997). However, the pre-antibiotic plasmid pMURTLE (a possible ancestor of all IncH plasmids), R478 (IncHI2) and MIP233 (IncHI3) all confer similar levels of RSH protection. Cells harbouring the IncHII plasmid pHH1508a and the subclone from pHH1457-2, pDAK, show thiol oxidation levels similar to those harbouring the IncHII subclone pMJ606 (Fig. 3), which only contains terABCDEF\textsuperscript{e}. It is not known how much of the ter gene cluster the IncHII plasmids contain.

The molar ratio of RSH oxidized to tellurite oxyanions accumulated (Turner et al., 1995a) is 0.8 ± 0.1 for tellurite-susceptible cultures. The ratio in Te\textsuperscript{e} cultures with high levels of thiol protection is 0.1 ± 0.1 (IncHI, IncP\textalpha and teh) and ranges from 0.2 to 0.3 (± 0.1) for the IncHII plasmids.

### Tellurite MICs of dsbA and dsbB mutants

The resistance levels of E. coli strains with mutations in genes reported to be involved in disulphide-bond formation (dsb) and effect of overexpressing these genes are shown in Table 2. The dsbA and dsbB mutants gave MICs of 0.008–0.0015 μg K\textsubscript{2}TeO\textsubscript{4} ml\textsuperscript{-1}, which is the greatest degree of hypersensitivity observed to date, more than that observed for nitrate reductase mutants (MIC 0.03 μg K\textsubscript{2}TeO\textsubscript{4} ml\textsuperscript{-1}) (Turner et al., 1995b). In

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**Table 2.** Tellurite resistance in strains with mutations in genes involved in disulphide-bond formation (dsb) and effect of overexpressing these genes

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype/plasmid\textsuperscript{a}</th>
<th>MIC (μg K\textsubscript{2}TeO\textsubscript{4} ml\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>R189</td>
<td>Wild-type</td>
<td>1–2</td>
</tr>
<tr>
<td>R190</td>
<td>dsbA</td>
<td>0.008–0.0015</td>
</tr>
<tr>
<td>R1317</td>
<td>dsbB</td>
<td>0.008–0.0015</td>
</tr>
<tr>
<td>R1179</td>
<td>dsbC</td>
<td>2–8</td>
</tr>
<tr>
<td>R1242</td>
<td>dsbD</td>
<td>2–8</td>
</tr>
<tr>
<td>R1361</td>
<td>dsbA, dsbD</td>
<td>1</td>
</tr>
<tr>
<td>R1318</td>
<td>dsbB, dsbD</td>
<td>0.25</td>
</tr>
<tr>
<td>R1385</td>
<td>dsbB, dsbC, dsbD</td>
<td>0.5</td>
</tr>
<tr>
<td>HB101</td>
<td>Wild-type</td>
<td>2</td>
</tr>
<tr>
<td>HB101</td>
<td>pSS18 (dsbA\textsuperscript{+})</td>
<td>0.25–0.5</td>
</tr>
<tr>
<td>HB101</td>
<td>pSS39 (dsbB\textsuperscript{+})</td>
<td>0.25</td>
</tr>
<tr>
<td>HB101</td>
<td>pSS18, pSS39 (dsbA\textsuperscript{+}, dsbB\textsuperscript{+})</td>
<td>0.25</td>
</tr>
<tr>
<td>R121†</td>
<td>p16-1 (dsbA\textsuperscript{+})</td>
<td>0.5–1</td>
</tr>
<tr>
<td>JCB819†</td>
<td>pCH1 (dsbB\textsuperscript{+})</td>
<td>0.5–1 (2)‡</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Plasmids pSS18 and pSS39 are described by Kishigami et al. (1995), p16-1 by Bardwell et al. (1991) and pCH1 by Guilhot et al. (1995).

†Strains R121 and JCB819 are dsbA and dsbB mutants, respectively.

‡The expression of DsbB in this construct is under control of the tac promoter and was induced with 0.1 mM IPTG. The MIC value reported in parentheses was obtained without the addition of IPTG.
The resistance levels were reduced in all three Te determinants studied. A similar resistance level was observed with the Te determinants studied. A similar resistance level was detected in dsbA or dsbB mutants harbouring Te determinants (results not shown).

**DISCUSSION**

The results described here present a significant step towards understanding the mechanism of tellurite toxicity and plasmid-mediated tellurite resistance. The data demonstrate that when E. coli is exposed to TeO$_4^{2-}$, there is a rapid decrease in the RSH content. This loss of RSH content (thiol oxidation) was tellurite dependent and not simply a result of cell death. Of the other toxins investigated, only Hg$^{2+}$ exposure caused a greater rate of cellular thiol oxidation. However, TeO$_4^{2-}$ exposure resulted in a larger molar concentration of oxidized thiol, suggesting that a wider range of RSH groups are accessible for reaction with tellurite, even though the Hg$^{2+}$ concentration was 100-fold greater than that of tellurite.

Mercury would catalyse the formation of metal thiol complexes, giving final reaction products of RSHg$^+$ and RSHgSR$^-$ (Means & Feeney, 1971). The reaction of Te(IV) compounds with the RSH nucleophile yields Te(SR)$_4^-$, which can undergo an oxidation/reduction disproportionation reaction giving Te(SR)$_2^+$ + RSSR. It is possible for Te(SR)$_2^+$ to react with further equivalents of RSH to produce RSSR and metallic tellurium (Albeck et al., 1998, and references therein). Tellurite was shown to cross-link the protein spectrum in erythrocyte membranes, which was considered to be the result of thiol–tellurium covalent bonds (Deuticke et al., 1992). No evidence of protein cross-linking was observed by comparing non-reducing SDS-PAGE profiles (results not shown). Te(IV) compounds have also been shown to selectively inhibit cysteine proteases (Albeck et al., 1998).

The RSH concentrations are maintained when Te$^+$ determinants from plasmids within the incompatibility groups IncPz, IncHII, IncHIII and IncHII are present. Overexpression of the E. coli chromosomal tehAtehB operon also confers protection against tellurite-dependent thiol oxidation. The protection of RSH oxidation afforded by the Te$^+$ determinants appears to be very efficient for there are only marginal losses of RSH content. None of the Te$^+$ determinants was able to protect against the mercury-dependent thiol oxidation or the oxidation generated by paraquat. This suggests that the proteins encoded by the Te$^+$ determinants interact with tellurite directly or are specific to the thiol–tellurium complex and not simply a general thiol:disulfide oxidoreductase or isomerase. This idea is further supported by the observation that the Te$^+$ determinants were unable to catalyse thiol:disulfide oxidation and thus were not replacing the DsbA activity to generate the resistance.

Hypersensitivity of E. coli to tellurite has been observed in nitrate reductase mutants (MIC 0.015–0.03 μg K$_2$TeO$_3$ ml$^{-1}$; wild-type MIC is 2 μg K$_2$TeO$_3$ ml$^{-1}$) (Avazeri et al., 1997). In addition to these very hypersensitive nar mutants, intermediate levels of sen-

### Table 3. Tellurite resistance mediated by Te$^+$ determinants within dsb mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (μg K$_2$TeO$_3$ ml$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>teh (pTWT100)</td>
</tr>
<tr>
<td>Wild-type</td>
<td>64–128</td>
</tr>
<tr>
<td>dsbA</td>
<td>32</td>
</tr>
<tr>
<td>dsbB</td>
<td>32</td>
</tr>
</tbody>
</table>
sitivity have been observed in mutants of oxidative stress metabolism (Turner et al., 1995b). Superoxide dismutase mutants are sensitive to 0.125–0.25 μg K$_3$TeO$_4$ ml$^{-1}$. Components of the thiol-disulfide redox system (thioredoxin, glutaredoxin, glutathione and their respective reductases) show sensitivity at 0.25–1 μg K$_3$TeO$_4$ ml$^{-1}$. In the present study we examined the effect on tellurite resistance of genes responsible for disulfide-bond formation in the periplasm (dsb).

Mutants affected in DsbA or DsbB were extremely hypersensitive to tellurite with MICs of $0.008–0.0015$ μg K$_3$TeO$_4$ ml$^{-1}$. However, mutants affected in other Dsb proteins did not show this hypersensitivity. Additionally, when dsbA or dsbB mutants were examined as double mutants with dsbD or dsbC, the high level of hypersensitivity was not observed. A possible explanation for these data considers that the DsbADsbB and DsbCDsbd pathways are thought to have different target substrates. Tellurite may react with the RSH groups of periplasmic proteins that are specifically oxidized by DsbA. Similar differences have been seen with mercury, cadmium, zinc and copper sensitivity in different dsb mutants and mutant combinations (Stafford et al., 1999; Rensing et al., 1997; Rietsch et al., 1996). Differential susceptibility of different dsb mutants was considered a result of binding of the metals to free thiols of specific classes of periplasmic proteins. Therefore, the hypersensitivity in the absence of DsbADsbB proteins suggests the possibility that these disulfide-bond catalysing enzymes may be involved in the folding of an enzyme which directly acts on tellurite oxidations or even more directly in the repair of tellurite-damaged protein thiols.

Alternatively, the differences in the susceptibility levels of the dsb mutants may arise from the differences in their proposed functions (reviewed by Missiakas & Raina, 1997). DsbA and DsbB are thiol-disulfide oxidoreductases, where DsbB oxidizes DsbA, allowing DsbA to catalyse the oxidation of cysteines of periplasmic targetted proteins. DsbD is involved in oxidizing DsbC and DsbE. DsbC has both thiol-disulfide oxidase activity (like DsbA) and disulfide isomerase activity. Therefore, from the sensitivity data of dsb mutants (Table 2), hypersensitivity is observed when the isomerase activity is present. When isomerase activity is missing (in dsbC and dsbD mutants) little to no sensitivity is observed. Together with the RSH oxidation data, this suggests that TeO$_2^{2-}$ reacts with RSH forming telluro-ether disulfide bonds (RS-Te-SR). Potentially, the isomerase (I) then acts on this bis-sulfotellurium group, giving rise to an inactivated target protein (TP) and/or isomerase–target protein complex. Potential reaction combinations may include:

$$\text{TeO}_2^{2-} + \text{TP(RSH)}_n \rightarrow \text{TP-RS-Te-SR-TP}$$

(1)

$$\text{TP-RS-Te-SR-TP + I} \rightarrow \text{TP-RS-Te-SR-I}$$

(2)

It is noteworthy that overexpression of the Dsb proteins did not give resistance, nor did they complement the hypersensitivity fully back to wild-type levels. This is in contrast to the overexpression of nitrate reductase, which generated moderate levels of resistance. Over-accumulation of the Dsb proteins may affect the balance with regard to any reactions that may involve tellurite and inadvertently aid the toxicity of tellurite.

At the present time a working model of tellurite toxicity envisages the oxyanion TeO$_2^{2-}$ entering the cells, likely via the phosphate uptake systems, and then being acted upon by the nitrate reductases. Tellurite which circumvents this line of defence may then react with the thiol redox equivalents within the cell. Along this pathway the Te$^0$ determinants must be acting either on the tellurite molecule directly, providing a form of detoxification, or, as mentioned above, on the products of Te(IV) chemistry, in order to repair the damage caused.

The mechanism of tellurite reduction has been recognized in a number of organisms. Micrococcus lactilyticus reduces inorganic oxyanions, including tellurite, with molecular hydrogen (Woolfolk & Whittley, 1962). NAD(P)H-dependent tellurite reductase activity has been observed in a number of organisms (Chiong et al., 1988; Moscoso et al., 1998; Terai et al., 1958). Rhodobacter spp. utilize tellurite and other oxyanions as terminal electron acceptors at an Fe–S protein (Moore & Kaplan, 1992), and nitrate reductase in E. coli possesses a tellurite reductase activity (Avazeri et al., 1997). Surprisingly, none of these mechanisms appears to incorporate thiol cofactor biochemistry, even though the present study and that of Albeck et al. (1998) establish that there is a high reactivity of Te(IV) with thiols. Additionally, cysteines have been identified to be key residues in the resistance mediated by the kilA determinant (Turner et al., 1994a).

It is clear that RSH and thiol biochemistry play an integral role in both wild-type and plasmid-mediated resistance in E. coli. Additionally, evidence is provided from the dsb mutant data that tellurite reactivity is likely more specific than initially thought. Not only are there key centres of tellurite reductase activity, but it is also likely that there is specific thiol:tellurium chemistry occurring at key target sites. It will be interesting to elucidate the specificity of the thiol components involved in tellurite biochemistry in both resistant and sensitive strains.

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