Tellurite-mediated disabling of [4Fe–4S] clusters of *Escherichia coli* dehydratases

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The tellurium oxyanion tellurite is toxic for most organisms and it seems to alter a number of intracellular targets. In this work the toxic effects of tellurite upon *Escherichia coli* [4Fe–4S] cluster-containing dehydratases was studied. Reactive oxygen species (ROS)-sensitive fumarase A (FumA) and aconitase B (AcnB) as well as ROS-resistant fumarase C (FumC) and aconitase A (AcnA) were assayed in cell-free extracts from tellurite-exposed cells in both the presence and absence of oxygen. While over 90 % of FumA and AcnB activities were lost in the presence of oxygen, no enzyme inactivation was observed in anaerobiosis. This result was not dependent upon protein biosynthesis, as determined using translation-arrested cells. Enzyme activity of purified FumA and AcnB was inhibited when exposed to an *in vitro* superoxide-generating, tellurite-reducing system (ITRS). No inhibitory effect was observed when tellurite was omitted from the ITRS. *In vivo* and *in vitro* reconstitution experiments with tellurite-damaged FumA and AcnB suggested that tellurite effects involve [Fe–S] cluster disabling. In fact, after exposing FumA to ITRS, released ferrous ion from the enzyme was demonstrated by spectroscopic analysis using the specific Fe²⁺ chelator 2,2'-bipyridyl. Subsequent spectroscopic paramagnetic resonance analysis of FumA exposed to ITRS showed the characteristic signal of an oxidatively inactivated [3Fe–4S]⁺ cluster. These results suggest that tellurite inactivates enzymes of this kind via a superoxide-dependent disabling of their [4Fe–4S] catalytic clusters.

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INTRODUCTION

Since no biological role of tellurium is known to date, scientific interest on it has focused mainly on its toxicity. Tellurite (TeO₃²⁻), one of the most oxidized forms of tellurium, is toxic to both prokaryotes and eukaryotes. Gram-negative bacteria are particularly sensitive to tellurium salts, whereas some Gram-positive species exhibit natural resistance to tellurite (Taylor, 1999). Experimental evidence accumulated during the last few years suggests that tellurite exerts its toxicity, at least in part, through the generation of reactive oxygen species (ROS) (Borsetti *et al.*, 2005; Calderón *et al.*, 2006; Pérez *et al.*, 2007; Tremaroli *et al.*, 2006).

Tellurite-mediated ROS generation was first suggested by studies with tellurite-hypersensitive *Escherichia coli* *sodAsodB* strains and by the observation that minimal

inhibitory concentrations of tellurite were higher for cells grown under anaerobic conditions (Tantaleán *et al.*, 2003). The leading ROS generated as consequence of tellurite reduction was shown to be superoxide (O₂⁻) (Calderón *et al.*, 2006). Recent work from our laboratory showed that tellurite-exposed *E. coli* exhibits increased cytoplasmic ROS, protein carbonyl content, thiobarbituric acid-reactive substances (TBARs), and superoxide dismutase (SOD) and catalase activities (Pérez *et al.*, 2007).

Various dehydratases involved in oxidative metabolism possess catalytic [4Fe–4S] clusters at their active sites that facilitate the binding and consequent dehydration of their substrates (Storz & Imlay, 1999). Solvent exposure of these clusters renders them vulnerable to superoxide, which is electrostatically attracted to the catalytic Fe atom of the cluster. Examples of such enzymes – which are commonly used as markers of oxidative damage – include fumarases A (FumA) and B (FumB) (Liochev & Fridovich, 1993), aconitase B (AcnB) (Gardner & Fridovich, 1991) and dihydroxyacid dehydratase (Storz & Imlay, 1999; Kuo *et al.*, 1987). Loss of Fe²⁺ by univalent oxidation of their [4Fe–4S]²⁺ centres results in the generation of a [3Fe–4S]⁺ cluster with a concomitant loss of enzyme activity (Imlay, 2003).

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Abbreviations: ITRS, *in vitro* tellurite-reducing system; MV, methyl viologen; ROS, reactive oxygen species; SOD, superoxide dismutase; TBAR, thiobarbituric acid-reactive substance.

Four supplementary figures are available with the online version of this paper.

In addition to FumA, FumB and AcnB *E. coli* encodes ROS-resistant isoforms fumarase C (FumC) and aconitase A (AcnA). Unlike FumA and FumB, FumC has no iron–sulfur centre and is fully resistant to superoxide. Its induction mediated by the SoxRS regulon allows some flux through the tricarboxylic acid cycle when FumA and FumB have been damaged by superoxide (Liochev & Fridovich, 1993). AcnA is also induced upon activation of *soxRS* under oxidative stress conditions, thus compensating for oxidative damage of the housekeeping AcnB (Varghese *et al.*, 2003).

We have previously observed that tellurite exposure results in decreased AcnB and FumA activities in *E. coli* (Pérez *et al.*, 2007). To shed further light on bacterial tellurite toxicity and because of the physiological relevance of this type of dehydratase, in this work we studied the effect of tellurite on [4Fe–4S]²⁺ cluster-containing enzymes using *E. coli* AcnB and FumA as models. Using genetic, chemical and biochemical approaches here we show that tellurite is particularly harmful for these dehydratases and that tellurite damage seems to be indirect. Enzyme inactivation appears to result from superoxide generation that occurs during intracellular tellurite reduction and involves Fe²⁺ release from the catalytic [4Fe–4S]²⁺ clusters.

METHODS

Bacterial strains and growth conditions. Bacterial strains used in this study are listed in Table 1. Bacteria were routinely grown in LB medium (Sambrook *et al.*, 1989) at 37 °C with vigorous shaking. Gene induction was carried out in the presence of 0.2 % arabinose. When needed ampicillin (100 µg ml⁻¹), chloramphenicol (50 µg ml⁻¹) or kanamycin (100 µg ml⁻¹) was added to the medium. Growth under anaerobic conditions used a nitrogen-purged Difco anaerobic chamber.

Enzyme activity. In general, enzyme activity was expressed as percentage activity, 100 % being the activity of the enzyme in extracts of cells not exposed to K₂TeO₃ at zero time. Total fumarase activity was determined by the formation of fumarate from L-malate at 250 nm for 2 min in a mix (1 ml) that contained 50 mM potassium phosphate buffer, pH 7.0, 50 µM L-malate and the extract (15 µg protein). FumC activity was measured after preincubating crude extracts for 2 h at

37 °C to inactivate endogenous FumA and FumB. A unit of FumC activity was defined as the amount of enzyme that converts 0.5 µmol L-malate min⁻¹ into fumarate at 25 °C (ϵ =1.62 mM⁻¹ cm⁻¹).

Aconitase activity was determined as the production of *cis*-aconitate from citrate at 240 nm as described by Varghese *et al.* (2003). A unit of aconitase activity was defined as the amount of enzyme that generates 0.5 µmol *cis*-aconitate min⁻¹ at 25 °C (ϵ =3.6 mM⁻¹ cm⁻¹).

qRT-PCR analysis. Total RNA was purified using the RNAsy kit (Qiagen). Briefly, *E. coli* was inoculated in 200 ml LB medium, grown to OD₆₀₀ ~0.6 and cultures were continued for 15 min after amending with potassium tellurite (0.5 µg ml⁻¹) or methyl viologen (MV, 100 µg ml⁻¹).

Two micrograms of purified RNA was used as template for qRT-PCR. Reactions were performed using the LightCycler RNA Amplification SYBR Green I kit (Roche Applied Science) as recommended by the vendor. Transcript amount (ng) for each gene was calculated from a standard curve made with known template concentrations. Specific primers used are listed in Table 2.

In vitro tellurite-reducing system (ITRS) and superoxide generation. The tellurite-reducing reaction mix consisted of 50 mM Tris/HCl buffer, pH 7.4, containing 1 mM K₂TeO₃, 1 mM NADH and 0.7 mg bovine liver catalase ml⁻¹ (Sigma). Standard reactions were incubated at 37 °C for 30 min as described earlier (Calderón *et al.*, 2006). A positive superoxide-generating control used 50 mM Tris/HCl buffer, pH 7.4, containing 0.05 mM xanthine (Sigma) and 1.6 mU xanthine oxidase ml⁻¹ (Sigma).

Cloning, overexpression and purification of FumA. The *E. coli* *fumA* gene was amplified by PCR (Table 2), purified, cloned in pET-TOPO/101 (Invitrogen) and transformed into *E. coli* TOP10. Purified recombinant plasmid pET-*fumA* was introduced into *E. coli* BL21(DE3) by electroporation.

E. coli BL21(DE3) carrying pET-*fumA* was induced with 1 mM IPTG for 5 h and FumA was purified by affinity chromatography on HisTrap HP (Amersham) columns according to the vendor's instructions. Protein concentration was determined by the method of Bradford (1976), using BSA as standard.

Reconstitution of tellurite-damaged [Fe–S] clusters. *E. coli* *fumC* or *acnA* strains were exposed to K₂TeO₃ (0.5 µg ml⁻¹) for 15 min, and crude extracts were prepared and incubated overnight under a N₂ atmosphere in the presence or absence of 150 µM NH₄Fe(SO₄)₂ prior to fumarase or aconitase activity assessment. Control extracts were prepared from unexposed cells.

Table 1. Bacteria used in this study

<i>E. coli</i> strain	Relevant genotype	Source or reference
BW25113	<i>lacI^A rrnB ΔlacZ hsdR514 ΔaraBAD ΔrhaBAD</i>	J. Imlay, University of Illinois, Urbana, USA
Top10	F ⁻ <i>mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 deoR nupG recA1 araD139 Δ(ara-leu)7697 galU galK rpsL(Str^R) endA1 λ⁻</i>	Invitrogen
OD570	BW25113 isogenic, <i>fumC</i> , Cm ^R	J. Imlay, USA
JWK0114	BW25113 isogenic, <i>acnB</i> , Km ^R	Nara Institute, Japan
JWK1268	BW25113 isogenic, <i>acnA</i> , Km ^R	Nara Institute, Japan
QC774	<i>sodA sodB</i> , Km ^R	Akiko Nishimura, Japan
PGS57	BW25113 isogenic, <i>fumA fumB fumC</i> ; carries the <i>fumA</i> gene cloned in pGS57 expression vector, Tet ^R	J. Imlay, USA
pET- <i>fumA</i>	<i>E. coli</i> BL21(DE3), transformed with pET-TOPO/101- <i>fumA</i> , Amp ^R	This work

Table 2. Primers used in this study

Primer	Orientation	Sequence (5'–3')
<i>fumCF</i>	Direct	GTCGTTCCCCATCACCTGACAGCA
<i>fumAR</i>	Reverse	GCGTAGCAGATGAACTGGCAGTCA
<i>acnAF</i>	Direct	GTTTCCATGCTTATCCCGGATGTA
<i>acnAR</i>	Reverse	TTCGACCAACTCGACCTGATCTTC
<i>csdB</i>	Direct	ATGTCTCCAACGTGCTTGGC
<i>csdBR</i>	Reverse	TTGGGTGTACCGGCTTCAAA
<i>soxSF</i>	Direct	TTACAGGCGGTGGCGATAATCGCT
<i>soxSR</i>	Reverse	CGAGCATATTGACCAGCCGCTTAA
<i>sodAF</i>	Direct	CCTGCCATCCCTGCCGTATGCTTA
<i>sodAR</i>	Reverse	TCGATAGCCGCTTTCAGGTCACCC
<i>rrsHF</i>	Direct	ATGACCAGCCACACTGGAAC
<i>rrsHR</i>	Reverse	TGACTTAACAAACCGCCTGC
<i>fumA</i> - TOPO	Direct	CACCATGTCAAACAAACCTTTCA
<i>fumA</i> - TOPO	Reverse	TTTACACAGCGGGTGC

Detection of free Fe²⁺ and EPR analysis. Purified FumA (15 µg) was exposed for 45 min to the ITRS, and 2,2'-dipyridyl (200 µM) was added to the mix. Formation of the 2,2'-dipyridyl-Fe²⁺ complex was monitored at 520 nm and released ferrous ion was estimated ($\epsilon=5.2 \text{ mM}^{-1} \text{ cm}^{-1}$) as described by Fu *et al.* (1994).

EPR spectra were obtained after treating purified FumA (in 20 mM potassium phosphate pH 7.4, 10% glycerol) for defined times with the ITRS or directly with 100 mM H₂O₂. Mixtures were frozen in quartz EPR tubes with liquid nitrogen and analysed as described by Djaman *et al.* (2004). H₂O₂ exposure was ended by addition of catalase (200 U ml⁻¹, final concentration).

RESULTS

Effect of tellurite on *E. coli* fumarase and aconitase activities

Aconitase and fumarase, two key metabolic enzyme activities, were used as models to study the effect of

tellurite on *E. coli* [4Fe–4S] cluster-containing dehydratases. ROS-sensitive FumA and AcnB and ROS-resistant FumC and AcnA were assayed in crude cell-free extracts from tellurite-exposed cells grown in the presence or absence of oxygen.

To assess the effect of tellurite on ROS-sensitive dehydratases, total fumarase and aconitase activities were determined in cell-free extracts from *E. coli fumC* and *E. coli acnA*, respectively. While fumarase and aconitase activities were inhibited by 80% in cells grown aerobically, no inhibitory effect was observed under anaerobic conditions (Fig. 1a, b). To rule out that the observed tellurite-mediated dehydratase inhibition could be due to a general blockage of the translation machinery, a control was carried out in which FumA activity was determined in extracts of chloramphenicol-treated *E. coli* exposed or unexposed to potassium tellurite (see supplementary Fig. S1, available with the online version of this paper). The same level of FumA was observed irrespective of chloramphenicol treatment, suggesting that the tellurite inhibitory effect is not related to enzyme translation.

FumC and AcnA were also assayed in cell-free extracts from *E. coli* exposed for defined times to K₂TeO₃. Extracts from wild-type cells previously treated to disable oxidation-sensitive FumA and FumB isoforms were used to analyse FumC, while extracts from *acnB* strains were used to analyse AcnA activity. Both FumC and AcnA activities increased in tellurite-treated cells as compared to unexposed controls (Fig. 2a, b). An approximately fourfold induction at 15 min post exposure was observed for both dehydratases. In addition, approximately 10% and 30% induction of *acnA* and *fumC* transcription, respectively, was observed in tellurite-exposed cells (Table 3). Increased transcription of two other genes involved in the oxidative stress response, *soxS* and *sodA*, was also observed in cells exposed to tellurite or MV (positive control). Interestingly, tellurite also strongly induced transcription of *sufS* (also known as *csdB*), which encodes a cysteine desulfurase

Fig. 1. Inhibition of *E. coli* ROS-sensitive dehydratases by K₂TeO₃. Total fumarase (a) and aconitase (b) activity was determined in cell-free extracts from *E. coli* cells exposed to K₂TeO₃ (0.5 µg ml⁻¹) for 30 min (Te) in aerobic (solid bars) and anaerobic conditions (open bars). C, unexposed control. Bars represent SD ($n=4$).

Fig. 2. FumC and AcnA induction in tellurite-exposed *E. coli*. FumC and AcnA specific activities were determined in cell-free extracts from *E. coli* wild-type and *acnB*, respectively. Cells were exposed for the indicated times to K_2TeO_3 ($0.5 \mu g\ ml^{-1}$, ●) or methyl viologen ($100 \mu g\ ml^{-1}$, ▲); ■, unexposed controls. Bars represent SD ($n=4$).

involved in repairing oxidatively damaged [Fe-S] clusters (Zheng *et al.*, 2001; Outten *et al.*, 2003). In contrast, the housekeeping *rrsH* gene (internal control), encoding 16S rRNA, showed a minor transcriptional repression in exposed cells (Table 3).

Reconstitution of tellurite-damaged aconitase and fumarase activities

Fumarase and aconitase reconstitution experiments were carried out using crude extracts of *E. coli* cells lacking the oxidative-resistant isoforms FumC and AcnA, respectively. Enzyme activities that had been damaged by tellurite *in vivo* were slightly restored upon overnight incubation of crude extracts in the absence of oxygen but fully recovered if Fe^{2+} ions were present (Fig. 3a, b). Aconitase [4Fe-4S] cluster reconstitution was hardly achieved – even in the presence of Fe^{2+} – in crude extracts of *E. coli* lacking *csdB*, a gene involved in the repair of [Fe-S] clusters (Fig. S2). Taken together, these results suggest that tellurite most probably affects these enzymes by disabling their [4Fe-4S] clusters.

Table 3. Tellurite-mediated transcriptional induction of anti-oxidant genes in *E. coli*

Gene	RNA (ng)		
	Control	K_2TeO_3 ($0.5 \mu g\ ml^{-1}$)	MV ($100 \mu g\ ml^{-1}$)
<i>soxS</i>	241 ± 11	438 ± 14	472 ± 14
<i>sodA</i>	338 ± 7	399 ± 12	410 ± 15
<i>fumC</i>	227 ± 9	290 ± 9	335 ± 7
<i>acnA</i>	310 ± 8	343 ± 6	370 ± 5
<i>csdB</i>	90 ± 16	193 ± 12	207 ± 10
<i>rrsH</i>	338 ± 8	315 ± 8	309 ± 6

In vitro tellurite reduction results in FumA inhibition

Purified *E. coli* FumA lost almost 90 % of activity when exposed to the ITRS (Fig. 4a). However, no effect was observed if the enzyme was treated with tellurite alone (not shown), a result suggesting that superoxide radical being produced during incubation of FumA with ITRS could be involved in the tellurite-mediated damage to the enzyme. To assess if superoxide is actually involved in FumA inhibition, a control was carried out in which purified FumA was incubated for 30 min with a known superoxide-generating system (xanthine/xanthine oxidase). Fig. 4(a) shows that FumA exposed to either superoxide-generating system lost ~90 % activity under aerobic conditions as compared to untreated controls. To obtain further evidence that superoxide is involved in FumA damage, the same experiment was carried out under anaerobic conditions. No significant differences in enzyme activity were observed in superoxide-exposed and control FumA in the absence of oxygen (Fig. 4b). Enzyme activity of the ITRS-damaged purified FumA was almost fully recovered after incubating in the presence of DTT and ferrous ion under anaerobic conditions (Fig. 4c), indicating that this *in vitro* FumA inhibition involves the disabling of its [4Fe-4S] cluster.

To confirm this last assumption ferrous ion released from purified FumA after exposure to the ITRS was assessed using the highly specific Fe^{2+} chelator 2,2'-bipyridyl. The enzyme lost over 90 % of its Fe^{2+} upon ITRS exposure as compared to unexposed controls (not shown), confirming that tellurite-mediated enzyme inactivation involves [4Fe-4S] cluster disruption. Further support for these findings came from EPR experiments in which purified FumA was incubated for 10 and 30 min with the ITRS or for 5 and 30 min with H_2O_2 (control). At short incubation times the characteristic signal corresponding to the paramagnetic, inactive $[3Fe-4S]^+$ cluster was observed (Fig. S3),

Fig. 3. Reconstitution of tellurite-damaged fumarase (a) and aconitase (b) activities in crude extracts of *E. coli* cells exposed for 15 min to K_2TeO_3 ($0.5 \mu g ml^{-1}$). C, unexposed control; Te, tellurite-exposed; Te/Ana, tellurite-exposed, incubated in anaerobic conditions for 12 h; Te/Ana/Fe, as Te/Ana, but in the presence of Fe^{2+} ions. Bars represent SD ($n=3$).

strengthening the idea of an oxidatively mediated loss of Fe^{2+} from the enzyme.

DISCUSSION

The ultimate molecular mechanism underlying bacterial tellurite toxicity is not fully understood. Preliminary evidence was obtained years ago from studies aimed at identifying and characterizing genetic bacterial tellurite resistance (Te^r) determinants. For example, the *cysK* gene of *Geobacillus stearothermophilus* V, encoding cysteine synthase, functions as a Te^r determinant in *E. coli* (Vásquez *et al.*, 2001). Other groups have also reported that expression of cysteine synthase genes from *E. coli* (Alonso *et al.*, 2000), *Rhodobacter sphaeroides* (O'Gara *et al.*, 1997), *Staphylococcus aureus* (Lithgow *et al.*, 2004) and *Azospirillum brasilense* (Ramírez *et al.*, 2006) conferred

resistance to potassium tellurite. It was shown that the *iscS* gene from *G. stearothermophilus* V, encoding a cysteine desulfurase, also conferred tellurite resistance in *E. coli* (Tantaleán *et al.*, 2003). In addition, *E. coli* lacking *csdA*, *csdB* or *iscS* was shown to be hypersensitive to K_2TeO_3 (Rojas & Vásquez, 2005). Cysteine synthase (CysK) and cysteine desulfurase (IscS) enzymes are probably functionally related since IscS catalyses the desulfurization of L-cysteine and participates in the incorporation of the released sulfur atom into ROS-damaged [Fe-S] clusters (Zheng *et al.*, 1993, 1994). Cysteine desulfurases from the *iscAUS* and *sufABCDSE* operons of *E. coli* are involved in a complex machinery responsible for *de novo* synthesis as well as for the repair of ROS-damaged [Fe-S] clusters (Djaman *et al.*, 2004; Loiseau *et al.*, 2003). Thus, it was tempting to speculate that repair of [Fe-S] clusters may represent a bacterial defensive mechanism against ROS elicitors such as potassium tellurite.

Fig. 4. Inhibition of FumA by an ITRS. Purified FumA was incubated for 30 min in aerobic conditions with ITRS (Te) or xanthine/xanthine oxidase (X/XO) in aerobic (a) and anaerobic conditions (b). (c) Reconstitution of tellurite-damaged purified FumA: FumA exposed to ITRS (Te); reconstitution in anaerobic conditions in the presence of dithiothreitol (DTT) and Fe^{2+} ions (Te/DTT/Fe). C represents unexposed FumA in all panels.

Previous results from our laboratory showed that tellurite-exposed *E. coli* exhibited decreased fumarase and aconitase activities and that *in vivo* and *in vitro* reduction of tellurite resulted in superoxide production (Calderón *et al.*, 2006; Pérez *et al.*, 2007). Since some of the main intracellular superoxide targets are [4Fe–4S] cluster-containing enzymes (Imlay, 2003), we speculated that superoxide generated as a consequence of tellurite reduction could be involved in inhibiting aconitase and fumarase. In this work the oxidizing ability of tellurite on AcnB and FumA was evaluated using *acnA* and *fumC* cells, respectively. The observed inhibition of AcnB and FumA was almost entirely dependent on the presence of oxygen, suggesting that tellurite's inhibitory effect was mediated by ROS (Fig. 1). Subsequent *in vitro* experiments demonstrated that tellurite-mediated FumA inactivation involves Fe^{2+} release from the enzyme, a common feature observed in ROS-damaged [4Fe–4S] clusters (Flint *et al.*, 1993a, b; Imlay, 2003).

To further demonstrate that tellurite reduction drives FumA inactivation, the enzyme was exposed to an ITRS (Calderón *et al.*, 2006; Pérez *et al.*, 2007). As a positive control, FumA was independently exposed to xanthine/xanthine oxidase, a well known superoxide-generating system. In both cases FumA activity was reduced over 90 % in the presence of oxygen, a result that was not observed under anaerobic conditions (Fig. 4a, b). The direct involvement of superoxide in FumA inactivation was demonstrated using SOD. Fig. S4 clearly shows that FumA is protected from ITRS inactivation in the presence of superoxide dismutase.

Increased intracellular superoxide levels result in augmented SodA activity, a consequence of SoxRS-mediated *sodA* transcriptional activation (Imlay, 2003). Although *sodA* induction was previously observed in tellurite-exposed *E. coli* (Pérez *et al.*, 2007) here we show that it occurs at the transcriptional level. Transcription of *acnA*, *fumC* and *sufS* was also found to be increased in tellurite-exposed cells (Table 3).

Brown *et al.* (2002) demonstrated that inactive forms of $[\text{4Fe-4S}]^{2+}$ clusters, $[\text{3Fe-4S}]^+$, are paramagnetic and can be detected *in vivo* by paramagnetic resonance spectroscopy (EPR). In this work, EPR was used to demonstrate the presence of the inactive $[\text{3Fe-4S}]^+$ form in purified FumA exposed to ITRS (Fig. S3). FumA exposure to tellurite (or H_2O_2 , positive control) for 30 min caused the characteristic signal at 3200 gauss (0.32 T) to disappear, which is most probably due to overexposure of the ROS-sensitive cluster generating non-paramagnetic structures (Djaman *et al.*, 2004).

Finally, it is worth making some comments about the high toxicity of K_2TeO_3 for micro-organisms. As was observed by Turner *et al.* (1999, 2001) tellurite causes thiol oxidation, compromising seriously the redox balance of the cell. Moreover, superoxide generated as byproduct of tellurite reduction (Pérez *et al.*, 2007) may result in increased intracellular H_2O_2 levels, either by specific

superoxide dismutation by SOD or by accidental transfer of electrons to O_2^- during auto-oxidation of respiratory dehydrogenases (Massey, 1994; Messner & Imlay, 1999, 2002). Since H_2O_2 also oxidizes [Fe–S] clusters (Storz & Imlay, 1999), released Fe^{2+} can react with H_2O_2 (Fenton reaction) to generate hydroxyl radical, of which nucleic acids and membrane lipids (Refsgaard *et al.*, 2000) are important cellular targets. In this context, increased TBARs have been found in *E. coli* exposed to potassium tellurite (Pérez *et al.*, 2008). Since oxidative stress results in widespread damage of the cell, an immediate consequence is a decrease in cell viability. Several tellurite resistance determinants identified so far seem to deal with adaptive responses of a cell facing oxidative conditions. Experiments to shed further light on these interesting findings are under way in our laboratory.

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