Biochemical and Biophysical Research Communications 380 (2009) 148–152

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Expression of *Aeromonas caviae* ST pyruvate dehydrogenase complex components mediate tellurite resistance in *Escherichia coli*

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ARTICLE INFO

Article history:
Received 22 December 2008
Available online 23 January 2009

Keywords:
K_2TeO_3
Tellurite
Pyruvate dehydrogenase complex
Aeromonas caviae
ROS
Reactive oxygen species

ABSTRACT

Potassium tellurite (K_2TeO_3) is harmful to most organisms and specific mechanisms explaining its toxicity are not well known to date. We previously reported that the *lpdA* gene product of the tellurite-resistant environmental isolate *Aeromonas caviae* ST is involved in the reduction of tellurite to elemental tellurium. In this work, we show that expression of *A. caviae* ST *aceE*, *aceF*, and *lpdA* genes, encoding pyruvate dehydrogenase, dihydrolipoamide transacetylase, and dihydrolipoamide dehydrogenase, respectively, results in tellurite resistance and decreased levels of tellurite-induced superoxide in *Escherichia coli*. In addition to oxidative damage resulting from tellurite exposure, a metabolic disorder would be simultaneously established in which the pyruvate dehydrogenase complex would represent an intracellular tellurite target. These results allow us to widen our vision regarding the molecular mechanisms involved in bacterial tellurite resistance by correlating tellurite toxicity and key enzymes of aerobic metabolism.

The low-abundance chalcogen element, tellurium (Te), belongs to the 16 (VIA) group of the periodic table and exhibits no toxicity in its elemental state (Te^0). However, its soluble oxyanions tellurite [TeO_3^{2-}, Te(IV)] and tellurate [TeO_4^{2-}, Te(VI)] are highly toxic both for prokaryotes and eukaryotes [1]. Tellurite is by far more toxic than tellurate, especially for Gram negative bacteria, even at concentrations as low as 4 μM [2]. Bacterial sensitivity to tellurium compounds is well documented. In fact, Alexander Fleming wrote about the antibacterial properties of potassium tellurite (K_2TeO_3) as early as 1932 and since then, tellurite has been used routinely as a selective agent in microbiological culture media [3].

TeO_3^{2-} resistance (Tel^R) determinants have been found in plasmids or the chromosome in different bacterial species which exhibit great variability of aminoacid/nucleotide sequences thus hampering the proposition of a general mechanism to explain the tellurite resistance/toxicity phenomenon. Some of the determinants identified so far play a role in diverse metabolic pathways such as *Escherichia coli* nitrate reductases NarG and NarZ, which are able of tellurite reduction and seem to be responsible for the basal resistance of this bacterium to tellurite [4]. Other enzymes exhibiting the ability to reduce tellurite are *Staphylococcus epidermidis* catalase [5] and dihydrolipoamide dehydrogenase from *Aeromonas caviae* and other bacteria [6]. In addition, *Geobacillus stearothermophilus* V *cysK* and *iscS* genes, encoding cysteine synthase and cysteine desulfurase, respectively, have been also shown to mediate tellurite resistance in *E. coli* [7,8]. Similar results were reported for the *Staphylococcus aureus cysM* gene [9].

Tellurite entry to the cell would occur most probably through phosphate carriers [10], although monocarboxylate [11] and iron [12] transporters could also be involved in TeO_3^{2-} entrance into the cell. Once inside the cell, tellurite would be partially reduced by nitrate reductase or other enzymes [4–6]. When tellurite concentration reaches levels high enough to overcome this barrier, important biomolecules are affected. One of the first intracellular tellurite targets to be identified was glutathione (GSH), whose levels significantly decrease upon tellurite exposure, affecting seriously the redox balance of the cell [13]. Later, Lohmeier-Vogel et al. [14] communicated that tellurite exposure compromises the cell metabolism by causing the loss of the transmembrane pH gradient and decreased cytoplasmic ATP levels in *E. coli*. In addition, it has recently been shown that *E. coli* [15] and *Pseudomonas pseudoalcaligenes* KF707 [16] exhibit a noticeable increase of intracellular reactive oxygen species (ROS) when exposed to potassium tellurite, indicating, at least in part, that tellurite exerts its toxic effects through the establishment of an oxidative stress status in the cell.

Regardless of the above considerations, the ultimate mechanism(s) underlying tellurite toxicity is(are) not known to date and it has been repeatedly argued that tellurite toxicity results from its strong oxidant character [2].

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doi:10.1016/j.bbrc.2009.01.078

We recently reported that chromatographic fractions containing the pyruvate dehydrogenase (PDH) complex of the tellurite-resistant environmental isolate *A. caviae* ST (hereafter *A. caviae*), in addition to PDH activity, exhibited tellurite reductase (TR) activity. Molecular cloning, expression studies, and purification of each PDH complex component allowed tracing TR activity to the E3 component, dihydrolipoamide dehydrogenase [6]. In this work we show that expressing *A. caviae aceE, aceF*, and *lpdA*, encoding pyruvate dehydrogenase, dihydrolipoamide transacetylase, and dihydrolipoamide dehydrogenase, respectively, mediate tellurite resistance in *E. coli*.

Materials and methods

Bacterial strains and culture conditions. Aeromonas caviae, *E. coli*, and their derivative strains used in this study are shown in Table 1. Cells were grown routinely at 37 °C in LB medium with constant shaking. When required, ampicillin (100 μg/ml), kanamycin (50 μg/ml) or chloramphenicol (25 μg/ml) was added to the medium.

Cloning and expression of A. caviae aceE, aceF, and lpdA genes. Genes encoding *A. caviae* component E1 (*aceE*), E2 (*aceF*), and E3 (*lpdA*) were amplified by PCR as previously described [6]. PCR products were cloned into pBAD TOPO® generating pBAE1, pBAE2, and pBAE3 recombinant plasmids which were subsequently introduced by electroporation into competent *E. coli* cells. Cells were grown in arabinose-amended LB medium to induce the expression of the respective proteins.

Detection of intracellular ROS. (a) H_2DCFDA (dihydro-dichlorofluorescein diacetate): *E. coli* was grown aerobically in arabinose-amended LB medium ($OD_{600} \sim 0.5$), washed with 10 mM phosphate buffer, pH 7.0 (buffer A), and exposed to sublethal tellurite concentrations (0.2 μg/ml) for 15 min. Cells were then incubated for additional 30 min in the presence of 20 μM H_2DCFDA (prepared in dimethyl sulfoxide). After washing twice with buffer A, cells were disrupted by sonication. Cell debris was eliminated by centrifugation for 10 min at 13,000*g* and fluorescence intensity was determined in an Applied Biosystems Citofluor 4000 Fluorescence Multi-well Plate Reader (excitation 410 nm, emission 519 nm) as described previously [15]. Results were normalized per mg of protein and expressed as percent fluorescence. (b) DHE (dihydroethidine): *E. coli* cells were grown as above, washed with 50 mM phosphate buffer, pH 7.4, centrifuged and suspended in the same buffer containing 0.2% glucose. Cells were incubated for 30 min at room temperature in the presence of potassium tellurite (0.2 μg/ml) and 127 μM DHE. Cell-free extracts were prepared and fluorescence intensity was determined (excitation 490 nm, emission 590 nm) as described earlier [17]. Results were normalized per mg of protein and expressed as percent fluorescence.

Determination of β-galactosidase activity. Cultures of *E. coli* carrying pBAD, pBAE1, pBAE2 or pBAE3 were grown in the presence of the inducer arabinose to an $OD_{600} \sim 0.3$ and then exposed to potassium tellurite (0.5 μg/ml) for 3 h at 37 °C with shaking as described previously [25]. OD_{600} was measured and cultures were chilled on ice. Then 100 μl of cell suspension were mixed with 800 μl of 60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, and 50 mM 2-mercaptoethanol, pH 7.0 and cells were permeabilized by adding chloroform and 0.1% SDS. The mix was preincubated for 10 min at 30 °C and after adding the substrate ONPG (*O*-nitrophenyl-galactoside, 0.73 mg/ml final concentration) it was incubated at 25 °C for 20 min. Reactions were halted placing the tubes for 10 min at 110 °C. Activity was expressed as Miller units per mg protein according to the procedure described by Miller [18].

Results

Clones of *E. coli* BW25113 carrying *A. caviae aceE, aceF* or *lpdA* genes cloned into pBAD expression vector were assessed for tellurite resistance by measuring growth inhibition zones in arabinose-amended agar plates. Expression of each *A. caviae* PDH complex component generated a Tel^R phenotype in *E. coli* BW25113. Indeed, each clone individually expressing the structural genes of *A. caviae* PDH complex exhibited reduced growth inhibition zones (~30%) as compared to the parental, isogenic strain, carrying the cloning vector alone (Fig. 1A). Similar results were obtained by determining tellurite minimal inhibitory concentrations (not shown). In turn, *E. coli* lacking *aceE*, *aceF* or *lpdA* genes were more sensitive to tellurite and showed growth inhibition zones $\geqslant 85\%$ than that of the parental, wild type strain. Genetic complementation of these mutants with the respective heterologous genes restored tellurite resistance levels to those of the control strain, again showing that the three genes of the PDH complex participate in K_2TeO_3 resistance (Fig. 1B).

Table 1
Bacterial strains used in this work.

E. coli strain/plasmid	Relevant genotype	Source or reference
BW25113	*lacI*^{q} *rrnB* Δ*lacZ hsdR*514 Δ*araBAD* Δ*rhaBAD*	Nara Institute, Japan
BW25113/pBAD	*E. coli* BW25113 carrying pBAD expression vector Ap^R	This work
BW25113/pBAE1	*E. coli* BW25113 carrying *aceE* from *A. caviae* ST cloned in pBAD (pBAE1) Ap^R	This work
BW25113/pBAE2	*E. coli* BW25113 carrying *aceF* from *A. caviae* ST cloned in pBAD (pBAE2) Ap^R	This work
BW25113/pBAE3	*E. coli* BW25113 carrying *lpdA* from *A. caviae* ST cloned in pBAD (pBAE3) Ap^R	This work
ADA110	*E. coli* AB734 λφ(*ibp::lacZ*) carrying pBAD Ap^R	Shapiro and Baneyx (2002)
ADA110/pBAD	Ap^R	This work
ADA110/pBAE1	Ap^R	This work
ADA110/pBAE2	Ap^R	This work
ADA110/pBAE3	Ap^R	This work
JW0110	*E. coli* BW25113 *aceE::kan*	Baba et al. (2006)
JW0111	*E. coli* BW25113 *aceF::kan*	Baba et al. (2006)
JW0112	*E. coli* BW25113 *lpd::kan*	Baba et al. (2006)
JW0110/pBAE1	Ap^RKan^R	This work
JW0111/pBAE2	Ap^RKan^R	This work
JW0112/pBAE3	Ap^RKan^R	This work
QC774	*E. coli* DE(*lac*)4169 *rpsL* DE(*sodA-lacZ*)49 DE(*sodB-kan*)1-DE(2) Cm^RKan^R	Nara Institute, Japan
QC774/pBAD	$Cm^RKan^RAp^R$	This work
QC774/pBAE1	$Cm^RKan^RAp^R$	This work
QC774/pBAE2	$Cm^RKan^RAp^R$	This work
QC774/pBAE3	$Cm^RKan^RAp^R$	This work