Microbiological Research 177 (2015) 15–21

Contents lists available at ScienceDirect

Microbiological Research

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

The ActP acetate transporter acts prior to the PitA phosphate carrier in tellurite uptake by *Escherichia coli*

Alex Elías[a,b,1], Waldo Díaz-Vásquez[a,b,1], María José Abarca-Lagunas[a], Thomas G. Chasteen[c], Felipe Arenas[a], Claudio C. Vásquez[a,*]

[a] Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile
[b] Facultad de Ciencias de la Salud, Universidad San Sebastián, Santiago, Chile
[c] Department of Chemistry and Texas Research Institute for Environmental Studies, Sam Houston State University, Huntsville, TX, USA

ARTICLE INFO

Article history:
Received 6 February 2015
Received in revised form 20 April 2015
Accepted 25 April 2015
Available online 8 May 2015

Keywords:
ActP
Tellurite uptake
Escherichia coli

ABSTRACT

The tellurium oxyanion tellurite is harmful for most microorganisms. Since its toxicity occurs chiefly once the toxicant reaches the intracellular compartment, unveiling the toxicant uptake process is crucial for understanding the whole phenomenon of tellurium toxicity. While the PitA phosphate transporter is thought to be one of the main paths responsible for toxicant entry into *Escherichia coli*, genetic and physiological evidence have identified the ActP acetate carrier as the main tellurite importer in *Rhodobacter capsulatus*. In this work, new background on the role of these transporters in tellurite uptake by *E. coli* is presented. It was found that, similar to what occurs in *R. capsulatus*, ActP is able to mediate toxicant entry to this bacterium. Lower reactive oxygen species levels were observed in *E. coli* lacking the actP gene.

Antioxidant enzyme catalase and fumarase C activity was almost unchanged after short exposure of *E. coli* Δ*actP* to sublethal tellurite concentrations, suggesting a low antioxidant response. In this strain, tellurite uptake decreased significantly during the first 5 min of exposure and inductively coupled plasma optical emission spectroscopy assays using an *actP*-overexpressing strain confirmed that this carrier mediates toxicant uptake. Relative gene expression experiments by qPCR showed that *actP* expression is enhanced at short times of tellurite exposure, while *pitA* and *pitB* genes are induced later. Summarizing, the results show that ActP is involved in tellurite entry to *E. coli* and that its participation occurs mainly at early stages of toxicant exposure.

1. Introduction

Tellurium (Te), a rather scarce element in the Earth's crust, has no known biological role to date. Occasionally Te is found as elemental tellurium (Te^0), but it most commonly forms alloys with metals like gold and silver. On the other hand, tellurium oxyanions tellurite (TeO_3^{2-}) and tellurate (TeO_4^{2-}) are well known for their toxicity (Taylor, 1999; Chasteen et al., 2009). The more soluble of these two, tellurite, is extremely noxious for microorganisms and has been used as selective agent in microbiology for decades (Fleming, 1932). In fact, regarding *Escherichia coli* minimal inhibitory concentrations, tellurite is by far more toxic than other metal(loid)s including mercury and silver (Nies, 1999).

However, the molecular foundation underlying tellurite toxicity is not completely clarified. At least part of it seems to be a consequence of its strong oxidant properties, which, to the end, results in the formation of reactive oxygen species (ROS) that damage cell macromolecules (Borsetti et al., 2005; Pérez et al., 2007; Calderón et al., 2009; Castro et al., 2008; Pradenas et al., 2013). On the other hand, reduced thiols like glutathione are among the main intracellular tellurite targets (Turner et al., 1999). Also, it has been suggested that upon tellurite exposure, Te can replace S and/or Se, thus inactivating defined proteins (Taylor, 1999; Cunha et al., 2009).

In spite of this, there are naturally-occurring tellurite-resistant bacteria that often reduce tellurite to elemental tellurium which accumulates as black intracellular deposits (Chiong et al., 1988; Moscoso et al. 1998), a process which could represent a first line of resistance/defense against the toxicant (Taylor, 1999). Along the same line, other bacteria detoxify tellurite by producing alkylated tellurium derivatives such as dimethyl tellurium and dimethyl ditelluride (Chasteen and Bentley, 2003; Araya et al., 2004; Chasteen et al., 2009).

* Corresponding author. Tel.: +56 2 2718 1117.
E-mail address: claudio.vasquez@usach.cl (C.C. Vásquez).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.micres.2015.04.010
0944-5013/

Currently, bacterial tellurite resistance is considered a multifactor response that involves, directly or indirectly, substrates and/or products of a number of metabolic pathways (Acuña et al., 2009; Castro et al., 2008, 2009; Valdivia-González et al., 2012). Given that tellurite exerts toxic effects only once it is inside the cell, it is mandatory to unveil the mechanism by which tellurite is transported from the extracellular space. In this context, while *E. coli* mutants lacking phosphate transport systems exhibited high tellurite tolerance (Tomás and Kay, 1986), alterations of the Pst phosphate transport system or the ChoQ protein resulted in a similar phenotype in *Lactococcus lactis* (Turner et al., 2007). Additional evidence showed that tellurite uptake is a pH-dependent process in *Rhodobacter capsulatus* (Borsetti et al., 2003). Later, the same group showed the active participation of the ActP monocarboxylate transporter in tellurite uptake (Borghese et al., 2008; Borghese and Zannoni, 2010). Finally, our group demonstrated that, at least in *E. coli*, tellurite transport is mediated mainly by the PitA phosphate transporter (Elías et al., 2012). In this work, the eventual participation of ActP was assessed in *E. coli.*

2. Materials and methods

2.1. Bacterial strains and growth conditions

E. coli BW25113 (*lacI*^{q} *rrnB*$_{T14}$Δ*lacZ*$_{WJ16}$ *hsdR*514 Δ*araBAD*$_{AH33}$Δ*rhaBAD*$_{LD78}$, (parental) and the isogenic, kanamycin-resistant, derivatives JW4028 (Δ*actP*), JW3578 (Δ*lacY*), JW2942 (Δ*glcA*), JW3898 (Δ*glpF*), JW3460 (Δ*pitA*) and JW2955 (Δ*pitB*) were from the KEIO collection of the National Institute of Genetics, Japan (Baba et al., 2006). *E. coli* AG1 (*endA*1 *recA*1 *gyrA*96 *thi*-1 *relA*1 *glnV*44 *hsdR*17 [$r_K^- m_K^+$], parental) and the ASKA derivatives that over-express cloned *actP* or *pitA* genes in the presence of 1 mM IPTG (Kitagawa et al., 2005) were obtained from the same source. Cell growth either in LB or M9 minimal medium supplemented with 0.2% glucose (Sambrook and Russell, 2001) was routinely started by inoculating pre-warmed fresh media with 1:100 (M9) or 1:1000 (LB) dilutions of overnight cultures. When required, kanamycin (10 μg ml^{-1}) or chloramphenicol (30 μg ml^{-1}) was added to the medium. Unless otherwise stated tellurite concentration was 20 μM. Growth was monitored at 600 nm in a TECAN INFINITE 200 PRO microplate reader.

2.2. Determination of growth inhibition areas and minimal inhibitory concentration

Bacteria were evenly spread in M9 minimal medium (containing 0.2% glucose) or LB agar (2%) plates amended with the appropriate antibiotic(s) when needed. Growth inhibition zones (GIZs) in the presence of defined metal ions were determined as described earlier (Fuentes et al., 2007).

Tellurite MIC was assessed in M9 minimal medium supplemented with 0.2% glucose. Aliquots (5 μl) of overnight cultures were mixed with 200 μl of medium deposited in 96-well microplates that contained increasing TeO_3^{-2}concentrations. Serial dilutions were performed starting with a sterile 400 μM solution. After overnight incubation at 37 °C, the minimal toxicant concentration inhibiting bacterial growth was determined.

2.3. Cell viability

Saturated cultures of *E. coli* BW25113 or mutant derivatives were diluted (1:100) with fresh M9-glucose medium and incubated at 37 °C with constant shaking to $OD_{600} \sim 0.05$. Cultures were again diluted 1:100 with the same medium (pre-warmed at 37 °C) and growth was continued to $OD_{600} \sim 0.15$. Then, TeO_3^{-2} was added (controls received sterile water) and at different time intervals aliquots were taken, diluted 10^6 fold, and plated in M9-glucose agar. After incubating overnight at 37 °C, the number of colony forming units (CFU) was determined.

2.4. Assessing ROS production

Total intracellular ROS and superoxide were determined using dihydrodichlorofluorescein diacetate (H_2DCFDA, λ_{ex} 490 nm–λ_{em} 522 nm) and dihydroethidine (DHE, λ_{ex} 520 nm, λ_{em} 610 nm), respectively. After tellurite exposure, cells were centrifuged at 8000 × *g* for 3 min at 4 °C and washed with 50 mM Tris–HCl buffer, pH 7.4. The pellet was suspended in 1 ml of the same buffer and shaken with the probes (20 μM) for 30 (H_2DCFDA) or 15 (DHE) min at 37 °C in the dark. Then, optical density and fluorescence were determined in the TECAN apparatus.

2.5. Preparation of crude protein extracts

Cultures were centrifuged at 10,000 × *g* for 10 min at 4 °C, washed 2X with 2 ml of 50 mM potassium phosphate buffer, pH 7.4 (buffer A), and suspended in 1 ml of the same buffer. After adding the protease inhibitor PMSF (1 mM, final concentration) cells were disrupted by sonication and the cell debris was discarded by centrifuging as above. The supernatant was considered the crude extract, and protein concentration was determined as described previously (Bradford, 1976).

2.6. Enzyme activity

2.6.1. Catalase

The enzyme was assayed for 2 min by monitoring H_2O_2 decomposition at 240 nm. The reaction mix (1 ml) contained 50 mM potassium phosphate buffer, pH 7.0 and 19.4 mM H_2O_2. The reaction was started with the crude extract (45 μg protein) as described elsewhere (Chen and Schellhorn, 2003).

2.6.2. Fumarase C

The enzyme activity was assessed by measuring the formation of fumarate from L-malate for 2 min at 250 nm using the crude extract (15 μg protein). A molar extinction coefficient of 1.62 mM^{-1} cm^{-1} was used (Liochev and Fridovich, 1992).

2.7. Extracellular tellurite concentration

E. coli grown to $OD_{600} \sim 0.15$ was treated with tellurite (20 μM) and aliquots were taken at various time intervals to determine remaining tellurite in the supernatant as described previously (Molina et al., 2010).

2.8. Te quantification by inductively coupled plasma optical emission spectroscopy (ICP-OES)

Cells previously exposed to tellurite (1/2 MIC) were washed, suspended in 1 ml of buffer A and sonicated gently. Samples were diluted 10-fold with 10% HNO_3 and the whole volume was used for Te determination using a Spectro CIROS Vision ICP-OES apparatus as previously described (Díaz-Vásquez et al., 2014a). A calibration curve was constructed with commercially-available tellurium standards (Sigma).

2.9. Relative gene expression

Total RNA was prepared from cells grown in LB medium and exposed to tellurite (1/2 MIC) for 5, 10 and 15 min using the Favorprep tissue total RNA purification mini kit (Favorgene) and quantified using the Quant-it Ribogreen Kit (Invitrogen). qRT-PCR