Tellurite uptake by cells of the facultative phototroph *Rhodobacter capsulatus* is a ΔpH-dependent process

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Abstract The uptake by light-grown cells of *Rhodobacter capsulatus* of the highly toxic metalloid oxyanion tellurite (TeO_3^{2-}) was examined. We show that tellurite is rapidly taken up by illuminated cells in a process which is inhibited by the protonophore carbonyl cyanide-*p*-trifluoromethoxyphenyl-hydrazone (FCCP) and by the K^+/H^+ exchanger nigericin. Notably, the light-driven membrane potential ($\Delta\psi$) is enhanced by $K_2TeO_3 \geq 200$ μM. Further, tellurite uptake is largely insensitive to valinomycin, strongly repressed by the sulfhydryl reagent *N*-ethylethylmaleimide (NEM) and competitively inhibited by phosphate. We conclude that tellurite is transported into cells by a ΔpH-dependent, non-electrogenic process which is likely to involve the phosphate transporter (PiT family).

Key words: Membrane potential; Metalloid oxyanion; Protonophore; Tellurite uptake; Tetraphenylphosphonium ion electrode; *Rhodobacter capsulatus*

1. Introduction

During the last three decades, tellurium compounds have been extensively used in applied chemistry, metallurgy, and electronic industry [1,2]. Consequently, the water-soluble oxyanion tellurite (TeO_3^{2-}), can be found in high concentrations in land and waters near sites of waste discharge of industrial manufacturing processes [2]. It has been reported that K_2TeO_3 is extremely toxic to mammalian cells [3] and microorganisms [4] at concentrations as a low as 1 μg/ml (4 μM). Notably, several phototrophic bacteria have been shown to be resistant to a variety of metal and transition metals, including K_2TeO_3, at concentrations as high as 2.7 mg/ml [5,6]. Most of the tellurite-resistant bacteria convert tellurite to elemental tellurium (Te^0) which is accumulated intracellularly as black inclusions [5,2] although tellurite resistance without Te^0 accumulation was observed in a few species (see [5]).

The facultative phototrophs *Rhodobacter capsulatus* B100 and *Rhodobacter sphaeroides* 2.4.1 are highly resistant to tellurite (minimal inhibitory concentrations on agar media of 250 and 150 μg/ml K_2TeO_3, respectively [7,8]) and light-grown cells accumulate crystallites of Te^0 [7,8]. Notably, plasma membrane vesicles isolated from *R. capsulatus* B100 cells grown in the presence of K_2TeO_3 were shown to catalyze both photosynthetic and respiratory electron transport activities [7]. The latter results demonstrate that light-grown cells of *R. capsulatus* B100 accumulate Te^0 with no drastic effects on bacterial cell viability. This conclusion, along with the scarcity of information on the mechanism of uptake by bacteria (see [2]), prompted us to investigate the way the oxyanion tellurite can enter into cells of *R. capsulatus*.

In this work we show that tellurite uptake occurs at concentrations up to 120–160 μM (30–40 μg/ml); at higher concentrations, K_2TeO_3 increases the light-driven membrane potential ($\Delta\psi$) affecting the ΔpH component of the electrochemical proton motive force ($\Delta\mu_{H^+}$). As the K^+/H^+ exchanger nigericin inhibits tellurite uptake, we conclude that this latter process is ΔpH dependent and non-electrogenic. We also show that tellurite uptake involves a transporter(s) totally inhibited by the sulfhydryl reagent *N*-ethylethylmaleimide (NEM).

2. Materials and methods

2.1. Bacterial strain

R. capsulatus B100 was grown anaerobically in the light (200 W cm^{-2}) at 30°C in RCV-malate minimal salt medium as described previously [9]. Cells were grown to early stationary phase (A_{660} = 1.2) and used within 6 h of harvesting.

2.2. Determination of potassium tellurite uptake

The quantitative determination of K_2TeO_3 was done with the reagent diethyldithiocarbamate (DDTC) as in [10]. In this assay, DDTC was incubated with tellurite concentrations in the range of 1–50 μg/ml at neutral pH to form a yellow solution. The absorbance was read at 340 nm. Assay conditions: *t*, 28°C; cells equivalent to 1 mg/ml of proteins or 30 μg/ml of bacteriochlorophyll (BChl); light intensity, 200 W cm^{-2}. Anaerobic medium (Gly–Gly 50 mM or phosphate buffer 0.1 M, $MgCl_2$ 2.5 mM, KCl 10 mM, pH 7.4) was flushed for 1 h with pure N_2 gas before use. Inhibitor concentrations: ad hoc experiments (not shown) were performed to determine the optimal concentrations of the different inhibitors used in this study (see also [11–13]).

2.3. Electrode measurements of tetraphenylphosphonium (TPP^+) accumulation ($\Delta\psi$ determination)

A polyvinylchloride membrane, selectively permeable to TPP^+, was constructed as described in [11]. An internal cell volume of 102 μl/μmol of BChl was assumed, according to Kell et al. [14].

2.4. Protein and BChl determinations

Protein content of the samples was determined by the method of

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Abbreviations: BChl, bacteriochlorophyll; DCCD, dicyclohexyl carbodiimide; DDTC, diethyldithiocarbamate; $\Delta\mu_{H^+}$, electrochemical proton gradient; DIDS, 4,4-diisothiocyanatostilbene-2,2-disulfonate; FCCP, carbonyl cyanide-*p*-trifluoromethoxyphenyl-hydrazone; NEM, *N*-ethylethylmaleimide; TPP^+, tetraphenylphosphonium ion

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Lowry [15] using bovine serum albumin (BSA) as a standard. The content of BChl was measured spectrophotometrically in acetone/methanol (7:2 v/v) extracts using an ε_{775} of 75 mM^{-1} cm^{-1} [16].

2.5. Polarographic measurements

Respiratory activities in intact cells were determined with a Clark-type oxygen electrode YSI 53 (Yellow Springs Instruments Inc., Yellow Springs, OH, USA) as detailed elsewhere [9].

3. Results

3.1. Tellurite uptake by light-grown cells of R. capsulatus

We have recently reported that light-grown cultures of *R. capsulatus* are resistant to K_2TeO_3 concentrations up to 50 μg/ml [7]. Growth in the presence of K_2TeO_3 caused the bacteria to turn black, indicative of the presence of Te^0, and we have also shown that Te^0 crystals are accumulated inside the cells where tellurite reduction occurs [7]. Here (Fig. 1a) the light-driven tellurite uptake kinetics by *R. capsulatus* cells as a function of variable tellurite concentrations (up to 25 μg/ml) are shown. Considering the dissociation constants of tellurous acid (3×10^{-3} and 2×10^{-8} for k_1 and k_2, respectively), tellurite at neutral pH should be mainly present in the form of $HTeO_3^-$ and TeO_3^{2-} with no Te^{4+} present due to its instability in water. Based on this, the Lineweaver–Burk plot shown in Fig. 1a (inset) is likely to indicate the apparent K_m of the $HTeO_3^-/TeO_3^{2-}$ uptake process (K_m of 20 μg/ml or 80 μM). Under our assay conditions the tellurite uptake rate was 1.6 ± 0.1 μg/min per mg of protein, a value which is several times higher than the rate reported for *Escherichia coli* cells harboring tellurite-resistant determinants [15]. Notably, no uptake was seen at $K_2TeO_3 \geq 50$ μg/ml (200 nmol/mg of protein) (not shown). Fig. 1b shows that after 5 min of illumination, valinomycin, a mobile carrier catalyzing the electrical uniport of K^+ and dissipating the $\Delta\psi$, poorly affects the tellurite uptake (25% inhibition). Conversely, a strong inhibition is seen in the presence of the electroneutral exchanger nigericin (one K^+ for one H^+) (90% inhibition) and/or the proton translocator carbonyl cyanide-*p*-trifluoromethoxyphenyl-hydrazone (FCCP) (95% inhibition). These results indicate that tellurite is mainly taken inside the cells by a ΔpH-dependent process. Table 1 summarizes the rates of tellurite uptake in the presence of ionophores and of several reagents of sulfhydryl (NEM and mersalyl), carboxyl (dicyclohexyl carbodiimide, DCCD) and nucleophilic (4,4-diisothiocyanatostilbene-2,2-disulfonate, DIDS) groups. The mitochondrial phosphate carrier inhibitor NEM was greatly affected the tellurite uptake (95%) whereas another sulfhydryl reagent, mersalyl and the well-known inhibitor of the anion exchanger in mammalian cells DIDS [12] were less effective (40–50% inhibition). DCCD, an inhibitor of the ATPase proton channel [13] but also of the redox complex QH_2:cytochrome *c* oxidoreductase [18] involved in $\Delta\mu_{H^+}$ generation, had a strong inhibitory effect (70%) on tellurite uptake.

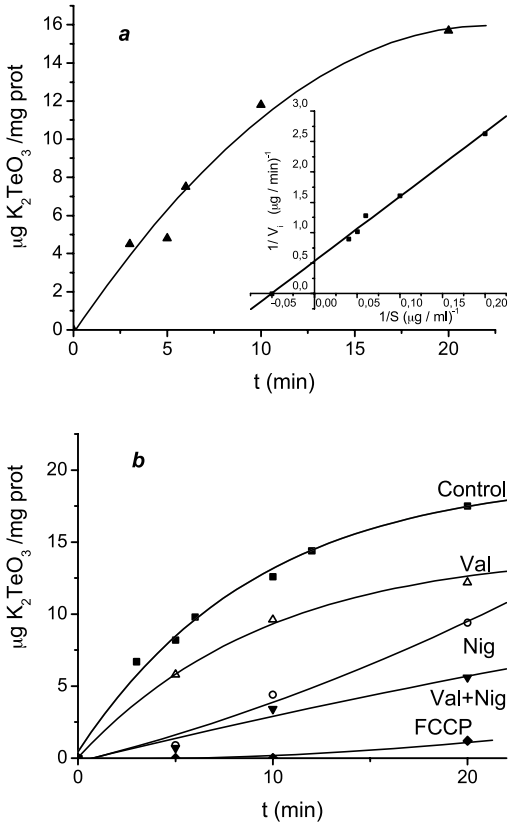

Fig. 1. a: Light-dependent tellurite uptake kinetic by *R. capsulatus* cells. Inset: Lineweaver–Burk plot. Assay conditions: $t = 28°C$, anaerobic Gly–Gly buffer (50 mM), $MgCl_2$ 2.5 mM, KCl 10 mM, pH 7.4. b: Tellurite uptake as a function of various ionophores: Val, valinomycin (4 μM); Nig, nigericin (3 μM); FCCP (5 μM). Assay conditions as in a except for the anaerobic buffer (phosphate 0.1 M). The apparent K_m was approximately 1 mM tellurite (not shown). See Section 2.2 for further details.

3.2. Tellurite effect on membrane potential generated by cells of R. capsulatus

To further verify the kinetic data of Fig. 1 indicating that tellurite uptake is not driven by the $\Delta\psi$, the distribution of the lipophilic cation TPP^+ was employed to estimate the $\Delta\psi$ in

Table 1
Effect of various ionophores and inhibitors on tellurite uptake

	TeO_3^{2-} uptake activity[a] (μg/min per mg of protein)	% of activity
Control	1.60 ± 0.08	100 ± 5
+FCCP (5 μM)	0.08 ± 0.03	5 ± 2
+Nigericin (3 μM)	0.16 ± 0.08	10 ± 5
+Valinomycin (4 μM)	1.20 ± 0.08	75 ± 5
+DIDS (50 μM)	0.80 ± 0.32	50 ± 20[b]
+DCCD (100 μM)	0.50 ± 0.08	30 ± 5
+NEM (0.5 mM)	0.08 ± 0.03	5 ± 5
+Mersalyl (0.2 mM)	0.95 ± 0.08	60 ± 5

[a]The uptake rates were determined on a 5 min time period of continuous illumination (see Section 2 and legend of Fig. 1b for details).
[b]The high value of statistical error is due to the optical interference at 340 nm between DIDS and the TeO_3^{2-} determination reagent (DDTC) (see also Section 2).

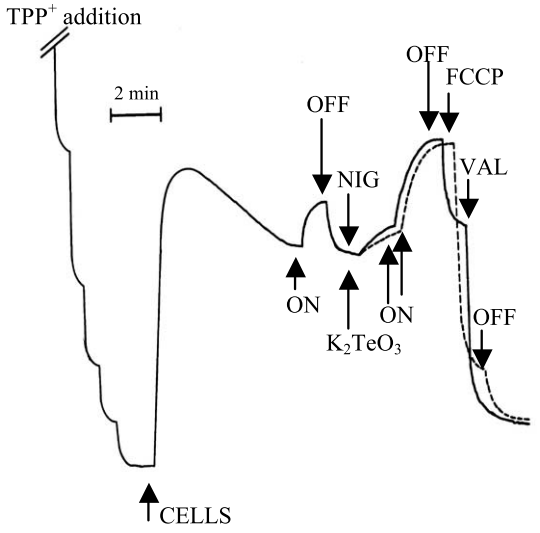

Fig. 2. Light-induced and oxygen-dependent uptake of TPP^+ ions by *R. capsulatus* cells. Following the calibration addition of TPP^+ (1 μM final concentration), cells (45 μg/ml of BChl) were added to 2 ml of air-saturated medium (TES buffer, 50 mM pH 7.5, KCl 10 mM, at 28°C). Symbols: ON, light-on; OFF, light-off. Abbreviations as in Fig. 1. See Section 2.3 and [11] for details.

cells of *R. capsulatus* under the same conditions used for the uptake kinetic measurements. Fig. 2 shows (continuous trace) that following the addition of *R. capsulatus* cells, a rapid upward deflection of the trace, indicative of TPP^+ uptake (development of a negative potential inside the cells), can be seen. Apparently, the initial level of $\Delta\psi$ cannot be maintained by respiration possibly due to the rate-limiting oxygen diffusion through the external membrane/wall structure of *R. capsulatus*. Under steady-state respiratory conditions (reached after approximately 5 min) the estimated $\Delta\psi$ was 104 ± 2 mV, while under both respiration and continuous illumination (light on), the $\Delta\psi$ went up to 137 ± 2 mV. Respiratory measurements, performed in parallel with TPP^+ uptake determinations, indicated that the endogenous respiration by cells of *R. capsulatus* is severely inhibited by light (80%, not shown). This suggests, in line with early reports [11], that the light-generated $\Delta\psi$ exhibits such a control over respiration that the latter process poorly contributes to $\Delta\psi$ formation when cells are subjected to continuous illumination. The zero $\Delta\psi$ was therefore defined by the TPP^+ uptake levels seen in the dark after the final addition of FCCP (1.5 μM). Fig. 2 also shows that addition of the K^+/H^+ exchanger nigericin (3 μM) increased the $\Delta\psi$ formed by both respiration and photosynthesis ($\Delta\psi = 147 \pm 2$ mV), through dissipation of the ΔpH component of the $\Delta\mu_{H^+}$. As expected, addition of valinomycin (4 μM) totally collapsed the TPP^+ uptake. Interestingly, also the addition of tellurite (50 μg/ml, i.e. 200 μM) increased the light-generated $\Delta\psi$ (interrupted trace), thus mimicking the effect of nigericin (see Section 4 for further discussion on this point).

4. Discussion and conclusions

This study examines the light-induced uptake of the metalloid oxyanion tellurite by cells of the facultative phototroph *R. capsulatus*. Results clearly indicate that the uptake is an energy-dependent process strongly repressed by the uncoupler FCCP and/or the K^+/H^+ exchanger nigericin but much less by the K^+ ionophore valinomycin. This suggests that the movement of tellurite from the external medium into cell cytoplasm, where tellurite reduction occurs [7], is an electroneutral mechanism driven mainly by the ΔpH. Tellurite uptake was repressed by the sulfhydryl reagent, phosphate carrier inhibitor NEM and it was partially sensitive to the oxyanion transporter inhibitor DIDS. Further, the apparent K_m for tellurite uptake varied from 80 μM to approximately 1 mM in the presence of 0.1 M phosphate (not shown). These observations, taken together, bring us to the conclusion that the uptake of tellurite by light-grown *R. capsulatus* is likely to be mediated by the phosphate transport system. In line with this it has previously been shown that the transport of phosphate (Pi) in *E. coli* is inhibited by tellurite [19]. Further, mutants of *E. coli* defective in Pi transport were resistant to levels of tellurite considerably higher (10–40 times) than those observed in most wild-type strains. Susceptibility to tellurite could be restored by a plasmid carrying the *phoB* region, which is involved in Pi regulation. Apparently, these early reports strongly support our proposal that *R. capsulatus* takes up tellurite by a Pi transporter, possibly of the PiT family [20]. In this respect, at neutral pH, we suggest the following generalized transport reaction:

$$n\mathrm{HTeO_3^-}\ (\mathrm{out}) + n\mathrm{H^+}\ (\mathrm{out}) \leftrightarrow n\mathrm{HTeO_3^-}\ (\mathrm{in}) + n\mathrm{H^+}\ (\mathrm{in})\ (1)$$

During the past decade, several determinants of tellurite resistance (Te^R) have been cloned and sequenced [8,17,21–23]. The chromosome of *E. coli* K12 contains two genes, named *tehA* and *tehB*, encoding for two polypeptides TehA and TehB. The TehA protein is highly hydrophobic [24] and it shows homology with the multidrug transporters of the SMR family. Although the connection between the phenotypes of cationic multidrug resistance and Te^R along with the biochemical nature of Te^R are not yet understood, the mechanisms of reduced uptake and/or efflux of TeO_3^{2-} have been ruled out [17]. As the latter conclusion is referred to *E. coli*, the mechanism of tellurite uptake here described for *R. capsulatus* can hardly be linked to the membrane polypeptides previously shown to be associated with Te^R in various bacterial species [2].

In conclusion, we believe that the present study not only contributes to a better understanding of how tellurite is taken up by light-grown cells of *R. capsulatus* but it also gives further insights into the multiple metabolic features of facultative phototrophs.

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