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ORIGINAL PAPER

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Interaction between uranium and the cytochrome c_3 of *Desulfovibrio desulfuricans* strain G20

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Abstract Cytochrome c_3 of *Desulfovibrio desulfuricans* strain G20 is an electron carrier for uranium (VI) reduction. When *D. desulfuricans* G20 was grown in medium containing a non-lethal concentration of uranyl acetate (1 mM), the rate at which the cells reduced U(VI) was decreased compared to cells grown in the absence of uranium. Western analysis did not detect cytochrome c_3 in periplasmic extracts from cells grown in the presence of uranium. The expression of this predominant tetraheme cytochrome was not detectably altered by uranium during growth of the cells as monitored through a translational fusion of the gene encoding cytochrome c_3 (*cycA*) to *lacZ*. Instead, cytochrome c_3 protein was found tightly associated with insoluble U(IV), uraninite, after the periplasmic contents of cells were harvested by a pH shift. The association of cytochrome c_3 with U(IV) was interpreted to be non-specific, since pure cytochrome c_3 adsorbed to other insoluble metal oxides, including cupric oxide (CuO), ferric oxide (Fe_2O_3), and commercially available U(IV) oxide.

Keywords Cytochrome *c* · *Desulfovibrio* · Uranium reduction

Introduction

Sulfate-reducing bacteria of the genus *Desulfovibrio* can reduce soluble uranium (VI) to insoluble uranium (IV) enzymatically (Lovley and Phillips 1992). Since this process has the potential for application in bioremediation efforts of contaminated environments, the parameters that delimit its functioning are of interest. The first step to understand the system for dissimilatory U(VI) reduction by *Desulfovibrio* is to identify the enzymatic machinery involved. The *c*-type cytochromes of *Desulfovibrio* are involved in U(VI) reduction. Previous work showed that cytochrome c_3 of *Desulfovibrio vulgaris* Hildenborough could act as a U(VI) reductase in a cell-free system, with hydrogen gas as the electron donor for U(VI) reduction (Lovley et al. 1993). This observation was confirmed by studies of a cytochrome c_3 mutant of *Desulfovibrio desulfuricans* strain G20. This mutant was essentially unable to utilize hydrogen gas as an electron donor for U(VI) reduction and was partially impaired when lactate or pyruvate was the source of electrons (Payne et al. 2002). These observations were also consistent with the model that some of the electrons from the cytoplasmic oxidation of lactate or pyruvate are utilized in the production of hydrogen gas or hydrogen equivalents, which are in turn oxidized by a periplasmic hydrogenase coupled to cytochrome c_3 (Noguera et al. 1998; Odom and Peck 1981). Furthermore, since the cytochrome c_3 mutant of *D. desulfuricans* G20 was still partially capable of U(VI) reduction with electrons from organic acids, it was inferred that at least one other cellular protein has the ability to act as a U(VI) reductase.

Since sulfate-reducing bacteria are found in significant numbers at uranium-contaminated sites (Abdelouas et al. 2000; Chang et al. 2001; Suzuki et al. 2003), their response to low levels of toxic metals might indicate an interaction that could affect reduction. The hypothesis was tested that exposure to a sub-inhibitory concentration of uranium might induce the cellular machinery of *D. desulfuricans* G20 responsible for the reduction of this metal. Prior exposure of *D. desulfuricans* G20 cells to uranium actually decreased the ability of those cells to reduce U(VI). Interestingly, growth of *D. desulfuricans* G20 in the presence of uranium did not affect the expression of the periplasmic cytochrome c_3; however, cytochrome c_3 was no longer among the soluble proteins from a high-pH wash of those cells. Instead, periplasmic cytochrome c_3 was found associated with precipitated uraninite. The ad-

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