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Short Communication

Electron acceptor dependence of electron shuttle secretion and extracellular electron transfer by *Shewanella oneidensis* MR-1

Chao Wu, Yuan-Yuan Cheng *, Bing-Bing Li, Wen-Wei Li, Dao-Bo Li, Han-Qing Yu

Department of Chemistry, University of Science & Technology of China, Hefei 230026, China

HIGHLIGHTS

- The secretion of flavins by *Shewanella oneidensis* MR-1 is differentially affected by electron acceptors.
- TMAO substantially stimulates the secretion of flavins by *Shewanella oneidensis* MR-1.
- Ferrihydrite reduction and current generation are enhanced in the presence of TMAO.

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ABSTRACT

Shewanella oneidensis MR-1 is an extensively studied dissimilatory metal-reducing bacterium with a great potential for bioremediation and electricity generation. It secretes flavins as electron shuttles which play an important role in extracellular electron transfer. However, the influence of various environmental factors on the secretion of flavins is largely unknown. Here, the effects of electron acceptors, including fumarate, ferrihydrite, Fe(III)-nitrilotriacetic acid (NTA), nitrate and trimethylamine oxide (TMAO), on the secretion of flavins were investigated. The level of riboflavin and riboflavin-5′-phosphate (FMN) secreted by *S. oneidensis* MR-1 varied considerably with different electron acceptors. While nitrate and ferrihydrite suppressed the secretion of flavins in relative to fumarate, Fe(III)-NTA and TMAO promoted such a secretion and greatly enhanced ferrihydrite reduction and electricity generation. This work clearly demonstrates that electron acceptors could considerably affect the secretion of flavins and consequent microbial EET. Such impacts of electron acceptors in the environment deserve more attention.

1. Introduction

Electron shuttles are the kind of redox-active compounds which can assist microbial extracellular electron transfer (EET). They play important roles in many microorganism-based applications, such as bioremediation and electricity generation in microbial fuel cell (MFC) (Rosenbaum et al., 2011). Attempts have been made for years to characterize the role of various electron shuttles in EET and further to utilize them for channeling electrons towards electron acceptors of our interests (Watanabe et al., 2009). For instance, antraquinone-2,6-disulfonate is able to transfer electrons from electrodes to dechlorinating bacteria to assist trichloroethene reduction (Aulenta et al., 2010). Bacteria can even secret electron shuttles by themselves to aid their respiration (Zhang et al., 2011).

Shewanella oneidensis MR-1 is an extensively studied model bacterium of *Shewanella* species, a group of dissimilatory metal-reducing bacteria. Most of *Shewanella* species can secret flavins, including riboflavin-5′-phosphate (FMN) and riboflavin, as electron shuttles (von Canstein et al., 2008). An electrochemical analysis revealed that approximately 70% electrons from *Shewanella* cells to electrodes were transferred by flavins (Marsili et al., 2008). Dose of FMN or riboflavin at a micromolar level was found to increase decolorization of naphthol green (Xiao et al., 2012) by *Shewanella* species. Apart from accelerating electron transfer from cells to electron acceptors, riboflavin was recently found to also reversely shuttle electrons from electrodes to cells (Ross et al., 2011), implying that electron shuttles might also assist electron transfer from some electron donors to cells.

All these findings suggest an essential role of flavins in EET of *Shewanella* species. It is well known that *S. oneidensis* MR-1 can use a wide range of electron acceptors through EET. However, up to date it remains unclear as to how electron acceptors influence the secretion of flavins. Thus, the primary goal of this study is to investigate the effects of several electron acceptors commonly found in subsurface and aquatic environments on the secretion of flavins by *S. oneidensis* MR-1, and to assess the consequent effects on EET.

* Corresponding author. Tel.: +86 05513606698; fax: +86 551 63606698.
E-mail address: chengyy@mail.ustc.edu.cn (Y.-Y. Cheng).

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2. Methods

2.1. Growth conditions of S. oneidensis MR-1 for the secretion of flavins and reduction of ferrihydrite

S. oneidensis MR-1 was routinely pre-cultured aerobically in Luria–Bertani medium until the stationary phase. Pre-cultured cells were collected by centrifugation and washed with basal medium (BM) (Campbell et al., 2006). Concentrated cultures were injected into sealed serum vials, each containing anaerobic BM with 20 mM lactate, to a final cell density of 2.0 at OD_{600}. The concentration of electron acceptors was 2 mM fumarate, 10 mM ferrihydrite, 2 mM Fe(III)-NTA, 2 mM nitrate and 2 mM TMAO, respectively. For ferrihydrite reduction, 10 mM ferrihydrite was added. TMAO of 0.02, 0.2 and 2 mM was respectively dosed, whereas riboflavin of 0.05, 0.5 and 5.0 μM was respectively dosed. No dose of TMAO or riboflavin was set as the controls.

The ferrihydrite was synthesized according to a previous report (Campbell et al., 2006). The reduction level of ferrihydrite was evaluated according to the concentration of reduced Fe(II) using ferrozine assay (Stookey, 1970).

2.2. Extracellular flavins analysis

At given time intervals, aliquots of cultures were collected from serum vials and centrifugated, and the flavins in the supernatants were detected. The concentrations of FMN and riboflavin were determined mainly following a method reported previously (von Canstein et al., 2008). After centrifugation (12000 rpm, 5 min), a 50-μL supernatant were injected into a liquid chromatograph (LC-1100, Agilent Inc., USA) equipped with C18 column (Waters Inc., Ireland). The mobile phase consisted of 25% methanol and 75% ammonium acetic acid (0.05 M, pH 7.0) in deionized water at a flow rate of 0.8 ml/min. Flavins were detected with an RF-10AXL fluorescence detector (Shimadzu Co., Japan) at an excitation wavelength of 420 nm and an emission wavelength of 525 nm. To obtain a calibration curve, standard solutions of FMN and riboflavin (Sigma Inc., USA) were prepared with BM at concentrations ranging from 0.1 to 10 μM. Concentrations of FMN and riboflavin in samples were determined by comparing the integrated area of the corresponding peak to the area of standard peaks.

2.3. Bioelectrochemical measurements

Chronoamperometric measurements were carried out on a CHI1030A electrochemical workstation (CH Instruments Co., China). An indium tin oxide glass was used as the working electrode and poised at +0.15 V (vs. Ag/AgCl). A platinum wire and a saturated Ag/AgCl electrode were used as the counter and reference electrodes, respectively. The electrolyte solution was prepared with anaerobic BM containing 20 mM lactate and 0.2 or 2 mM TMAO. Overnight cultures were collected and washed three times and injected into the electrochemical cells.

3. Results and discussion

S. oneidensis MR-1 can secret two types of flavins, FMN and riboflavin. The extracellular levels of FMN and riboflavin in the presence of different electron acceptors were measured. Considering that the bacterial growth rate might vary with different electron acceptors, stationary-phase cultures after being concentrated to a high cell density were used to minimize the growth and its consequent effect on the secretion of flavins. The results show that, in the presence of fumarate and after 12-h incubation, riboflavin secreted by cells reached to a submicromole level and FMN to a nanomole level (Fig. 1A and B).

The concentration of secreted flavins varied substantially with different electron acceptors. Compared with fumarate, nitrate showed an obvious inhibitory effect on the secretion of flavins (Fig. 1A and B). Nitrate has been previously reported to inhibit Fe(III) reduction (Cooper et al., 2003), an activity closely related to the function of flavins. Thus, the decreased secretion of flavins caused by nitrate might partially explain the previous observations.

Ferrihydrite suppressed the secretion of FMN and riboflavin, while Fe(III)-NTA had a stimulation effect. The Fe(III)-NTA, attributed to its high solubility, can be rapidly reduced by *S. oneidensis* MR-1, which result in a higher rate of energy generation compared with ferrihydrite. This likely explain the stimulation effect of Fe(III)-NTA on the secretion of flavins, which might be an energy-dependent process. Although from a theoretical calculation it was estimated that during lactate oxidation less than 1% ATP was required to synthesize flavins at levels detectable in a bioelectrochemical system, this did not include the energy required for the secretion of flavins (Marsili et al., 2008). A recent study revealed that a protein belonging to the multidrug and toxin efflux transporter family played an essential role in secreting flavins by *S. oneidensis* MR-1 (Kotloski and Gralnick, 2013). An efflux protein from this family has been demonstrated to function in an energy-dependent manner (Braibant et al., 2002). Thus, a further investigation into the energy requirement for the secretion of flavins would shed light on the differential effects of electron acceptors on the process. Moreover, different electron acceptors might also cause distinct genetic regulations and consequently affect the secretion

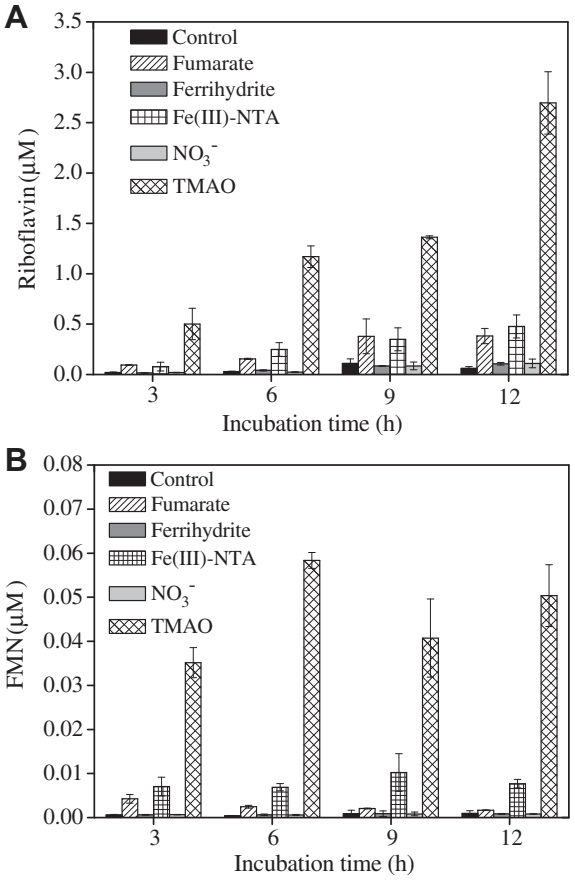

Fig. 1. Effects of alternative electron acceptors on the secretion of riboflavin (A) and FMN (B) by *S. oneidensis* MR-1. No electron acceptors addition was set as controls.

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