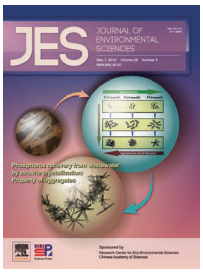
Journal of Environmental Sciences 26 (2014) 1118–1124

Available online at www.sciencedirect.com

Journal of Environmental Sciences

www.jesc.ac.cn



Humic acid-enhanced electron transfer of *in vivo* cytochrome c as revealed by electrochemical and spectroscopic approaches

Jiahuan Tang[1,2], Yi Liu[3], Yong Yuan[2], Shungui Zhou[2,*]

1. Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China. E-mail: 9841150@qq.com

2. Guangdong Institute of Eco-environmental and Soil Sciences, Guangzhou 510650, China

3. College of Bioscience and Biotechnology, Hunan Agricultural University, Changsha 410128, China

ARTICLE INFO

Article history:
Received 13 June 2013
revised 31 October 2013
accepted 07 November 2013

Keywords:
cytochrome c
humic acid
in vivo
in vitro
extracellular electron transfer

DOI: 10.1016/S1001-0742(13)60518-5

ABSTRACT

Out-membrane cytochrome c (Cyt c) plays an important role carrying electrons from the inside of microbes to outside electron acceptors. However, the active sites of Cyt c are wrapped by non-conductive peptide chains, hindering direct extracellular electron transfer (EET). Humic acids (HA) have been previously proven to efficiently facilitate EET. However, the inherent mechanism of HA-stimulated EET has not been well interpreted. Here, to probe the mechanism behind HA-stimulated EET, we studied the interaction between Cyt c and HA. The attachment of active *in vivo* Cyt c on a graphite electrode was achieved when MR-1 cells were self-assembled on the electrode surface. Pure horse-heart Cyt c was covalently immobilized on an electrode via 4-aminobenzoic acid to create an active *in vitro* Cyt c-enriched surface. Cyclic voltammetric measurements and scanning electron microscopy confirmed the immobilization of bacterial cells and pure Cyt c protein. Electrochemical methods revealed that HA could enhance the electrocatalytic current of both *in vitro* and *in vivo* Cyt c towards oxygen and thiosulfate, suggesting enhanced EET. The blue-shifted soret band in the UV-Vis spectra and changes in the excitation/emission matrix fluorescence spectra demonstrated that Cyt c interacted with HA to form organic complexes via electrostatic or hydrogen-bonding interactions. The results will help understand electron shuttle-stimulated EET and develop bacteria-based bioremediation and bioenergy technologies.

Introduction

A recent discovery that certain bacteria can transfer electrons produced from metabolic reactions directly to extracellular insoluble acceptors has created a new unexpected interest in biogeochemical cycles, biocorrosion, bioremediation and bioenergy (Bond et al., 2002; Videla and Herrera, 2009; Nielsen et al., 2010; Yuan et al., 2010). This type of electron transfer has been termed extracellular electron transfer (EET). *Shewanella* and *Geobacter*, for example, are two types of microbes that are capable of transferring electrons to extracellular electron acceptors. Three principal EET mechanisms have been proposed, that is, direct electron transfer via outer membrane (OM) redox proteins (Kim et al., 2002), direct electron transfer via electrically conductive pili (also known as "nanowires") (Reguera et al., 2005) and indirect electron transfer via electroactive metabolites (Rabaey et al., 2005). Direct electron transfer is considered to be more favorable for practical applications due to its higher efficiency. Previous investigations on direct EET to solid acceptors have revealed the important role of OM cytochromes c (Cyt c). For example, the OM MtrC (also known as OmcB) and OmcA have been shown to be involved in the *Shewanella oneidensis* MR-1-mediated reduction of Fe(III) oxides, and OmcE, OmcS and possibly OmcT in *Geobacter sulfurreducens*-mediated EET (Shi et al., 2009).

* Corresponding author. E-mail: sgzhou@soil.gd.cn

Humic acids (HA), ubiquitous in natural environments, are mixtures of high molecular-weight compounds (e.g., proteins, carbohydrates, lignin, aliphatic polymers) primarily derived from the degradation of plant and microbial materials. HA are generally considered recalcitrant under anaerobic conditions, but it was recently discovered that HA play a significant biological role. Numerous studies have revealed that HA can mediate electron transfer from microorganisms to iron (III) minerals or other spatially distant electron acceptors that are not directly accessible (Lovely et al., 1996; Jiang and Kappler, 2008; Wolf et al., 2009). HA also play an important role in carbon biogeochemistry, coupling terrestrial and aquatic carbon pools. For instance, HA extracted from a variety of wetland soils could act as either electron donors or electron acceptors and alter the ratio of greenhouse gases, suggesting the important role of HA in anaerobic decomposition (Keller et al., 2009). It has been speculated that the redox properties of HA are mainly attributed to quinone moieties (Scott et al., 1998). Previous studies have been focused on the electrochemical properties of HA derived from different sources and their efficiency in mediating EET (Lovley and Blunt-Harris, 1999; Peretyazhko and Sposito, 2005; Roden et al., 2010). However, very little is known about the mechanism behind HA-mediated EET from microbes to solid acceptors.

In this study, we hypothesized that the HA-mediated EET potentially arose from the interfacial interaction between HA and Cyt c, which serves as a short-term electron sink. Specifically, we used electrochemical methods to investigate the electron transfer process between Cyt c (both *in vivo* and *in vitro*) and HA. Furthermore, spectroscopic approaches were employed to reveal the *in vitro* and *in vivo* interactions between HA and Cyt c.

1 Materials and methods

1.1 Chemicals

Both Cyt c from horse-heart and HA (53680 humic acid) were obtained from Sigma Aldrich (USA). 4-Aminobenzoic acid (4-ABA) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Aldrich (USA). 4-ABA solution was freshly prepared for electrode modification. HA solution (0.1 mg/mL) was prepared by dissolving 0.01 g HA in 100 mL NaOH (0.1 mol/L). After HA completely dissolved, the solution was filtered through a 0.45 μm sterile membrane (GN-6 Metrice, Gelman Sciences, Ann Arbor, USA). Then the pH was adjusted to 7.0 by adding HCl to form a natural HA colloid. A fresh stock solution of 0.1 mg/mL HA was prepared daily.

1.2 Preparation of *in vitro* C-type cytochrome electrode

Prior to electrochemical modification, a glassy carbon (GC, 3 mm in diameter) electrode was polished with 1.0-, 0.3- and 0.05-μm α-Al_2O_3 powders successively and sonicated in water for about 5 min after each polishing step. After that, the GC electrode was first sonicated in water, then in ethanol, washed with ethanol and dried with a high purity nitrogen stream immediately before use. Electrochemical modification of the GC electrode was performed in 0.1 mol/L KCl solution containing 1 mmol/L 4-ABA, with potential scanning between +0.5 and +1.2 V (vs. Ag/AgCl) (Yang et al., 2006). After being rinsed with fresh deionized water, the electrode was immersed in a solution containing 200 mg/L Cyt c and 100 mg/L EDC in a phosphate buffer (pH 7.0) for 1 hr. Then, the prepared Cyt c-modified electrode was again rinsed in deionized water to remove the physically adsorbed Cyt c before electrochemical studies.

1.3 Preparation of *Shewanella oneidensis* MR-1 electrode

Shewanella oneidensis MR-1 was cultured in 50 mL of LB broth with gentle shaking (100 r/min) for approximately 18 hr until the optical density (OD_{600}) reached 2.0. Bacterial culture (1 mL) was inoculated into the sterilized electrochemical cell with 9 mL fresh medium. Bioelectrochemical experiments were carried out under potentiostatic control (CHI660D, Instruments, Inc., China) utilizing a three-electrode arrangement with a graphite plate working electrode (2.5 cm^2), a Ag/AgCl reference electrode and a Pt mesh counter electrode. After inoculation, the electrolyte was aseptically purged with nitrogen gas and the working electrode was poised at 0 V (vs. Ag/AgCl).

1.4 Analytical methods

Cyclic voltammetric measurements were performed with the modified GC or bacteria-attached electrode as working electrode. Electrochemical impedance spectroscopy (EIS) measurements were performed in the presence of 1 mmol/L $K_3[Fe(CN)_6]$/$K_4[Fe(CN)_6]$ (1:1) and 100 mmol/L KCl by applying an AC voltage (CHI660D, Instruments, Inc., China) with an amplitude of 5 mV and a frequency range of 0.1–100 kHz. All experimental solutions were deoxygenated by bubbling nitrogen for 15 min prior to measurements, and a nitrogen atmosphere was kept over the solutions during measurements.

Scanning electron microscope (SEM) images were obtained using an S-3700N scanning electron microscope (Hitachi, USA) at an acceleration voltage of 10 kV. UV-vis spectra of *in vitro* Cyt c were obtained with a TU-1810 spectrophotometer (Persee, China). UV-Vis spectra of *in vivo* Cyt c in strain MR-1 were performed in the diffuse transmission mode with bacterial cells suspended in a

phosphate buffer (Esteve-Núñez et al., 2008). The cell suspension was injected into a cuvette, which was mounted in front of an integrating sphere to measure the diffuse transmission light. Three-dimensional excitation/emission matrix (EEM) fluorescent spectra of the Cyt c, HA and the mixture of Cyt c and HA were recorded with a fluorescence spectrophotometer (Model F-4600, Hitachi, Japan) equipped with a 150 W xenon arc lamp as the excitation source. Spectra were recorded using 10 nm excitation and emission slits with the wavelengths in the range of 200 to 600 nm for both excitation and emission. Sigma Plot software was used to obtain the EEM spectra.

2 Results and discussion

2.1 Characterization of the Cyt c-attached electrodes

The attachment of active Cyt c on a graphite electrode was achieved when MR-1 cells self-assembled on the electrode surface. SEM was performed to confirm the attachment of MR-1 cells on the electrode. As shown in **Fig. 1a** and **b**, rod-shaped MR-1 cells were attached on the rough surface of the graphite plate. CV scans suggested the presence of active Cyt c after cell attachment. A pair of redox peaks

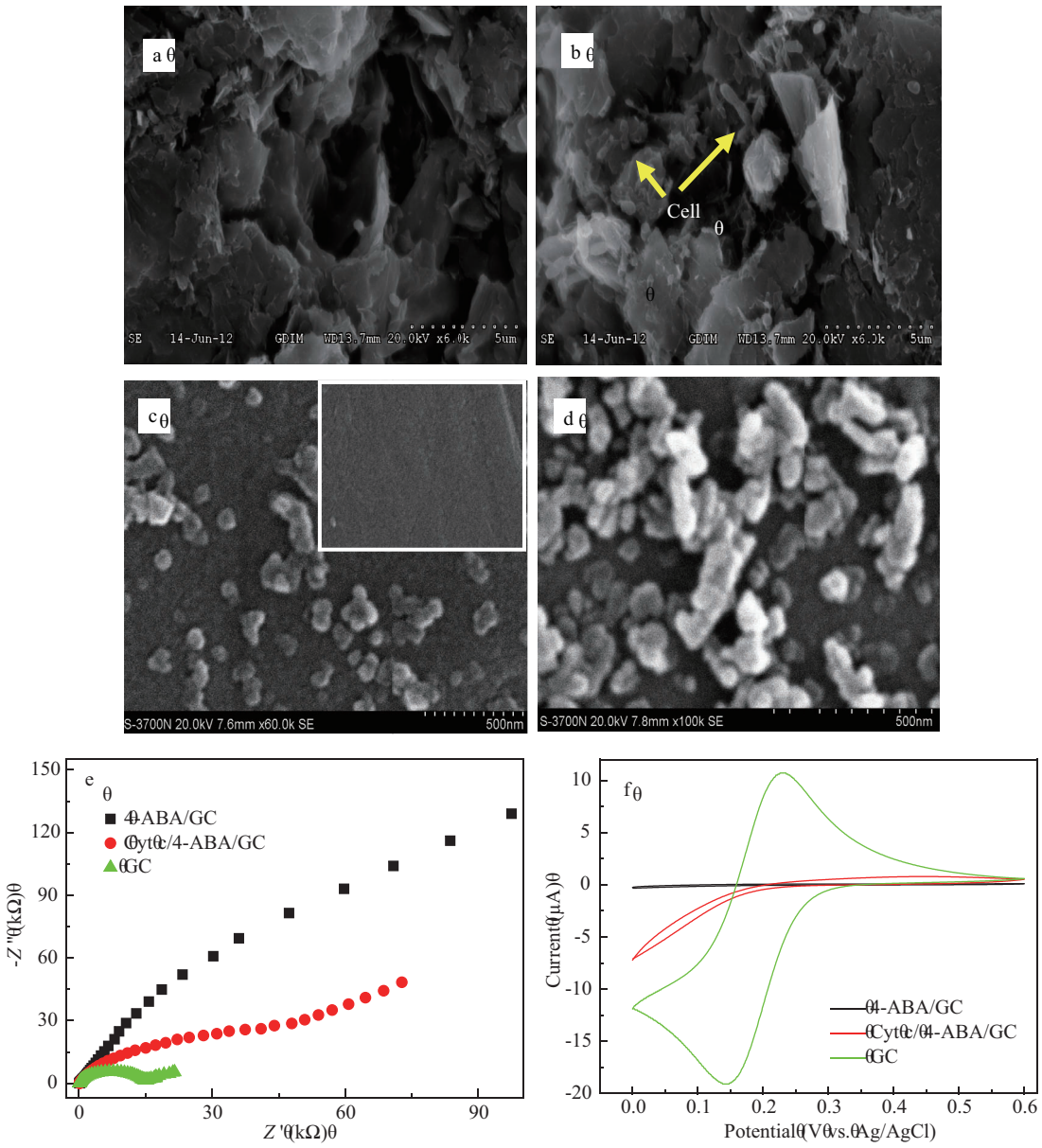


Fig. 1 SEM images of graphite plate before inoculation with *Shewanella oneidences* MR-1 (a) and MR-1 attached graphite plate (b); SEM images of 4-ABA modified GC electrode (c) (inset: blank GC electrode) and Cyt c modified GC electrode (d); Nyquist plots for 4-ABA modified and Cytc/4-ABA modified GC electrodes in the 0.10 mol/L KCl solution containing $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (both 2.5 mmol/L) as redox probe (e); CV scans of 4-ABA modified and Cyt c/4-ABA modified GC electrodes in N_2-saturated 0.10 mol/L KCl solution containing 2.5 mmol/L $K_3[Fe(CN)_6]$ and 2.5 mmol/L $K_4[Fe(CN)_6]$ at a scan rate of 100 mV/sec (f).

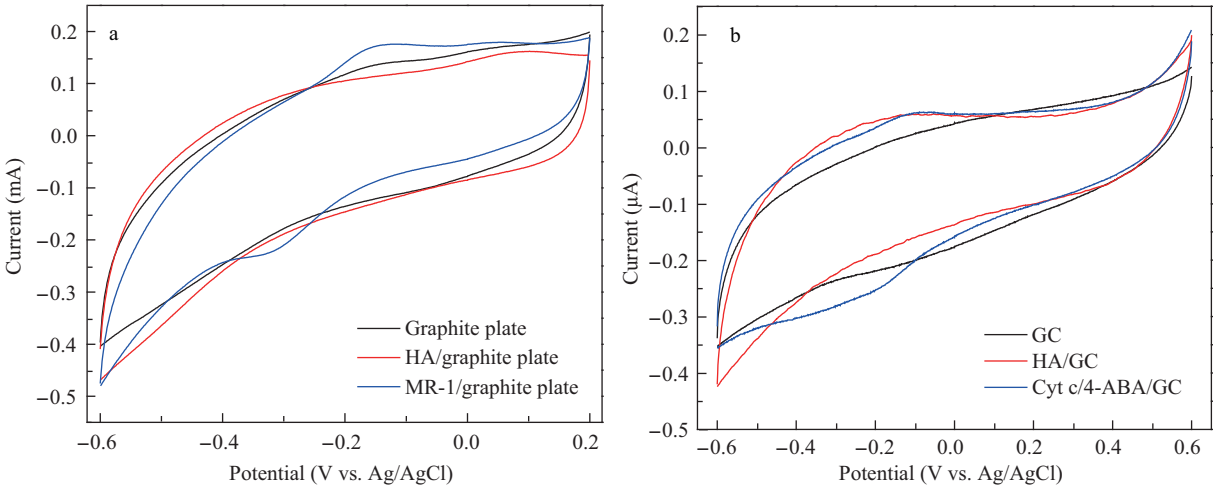


Fig. 2 CV scans of MR-1 attached electrode at a non-turnover state (a) and Cyt c/4-ABA modified electrode in N_2-saturated PBS (pH 7.0) solution (b) (scan rate: 10 mV/sec).

appeared on the cyclic voltammogram (CV) of the cell-attached electrode in the non-turnover state with a formal potential at −0.23 V (vs. Ag/AgCl) (**Fig. 2a**), identical to the CV feature of MR-1 reported in literature (Peng et al., 2010). Baron et al. (2009) suggested that both Mtrc and OmcA contributed to the redox peaks on the CV. The density of the electrochemically active Cyt c was calculated to be 1.0×10^{-10} mol/cm^2 on the graphite plate surface.

The immobilization of pure horse-heart Cyt c was confirmed by SEM and electrochemical measurements as well. Prior to the immobilization of Cyt c, the GC electrode was first covered with 4-ABA using an electrochemical method (Yang et al., 2006). Thereafter, Cyt c was covalently linked to the 4-ABA with EDC acting as the linking molecule. As shown in **Fig. 1c and d**, 4-ABA colloids were integrated on the surface of the GC electrode with potential scanning from +0.5 to +1.2 V (vs. Ag/AgCl), and after reacting with Cyt c, the size of the colloids apparently increased, suggesting successful immobilization of Cyt c. The surface modification process was also confirmed by EIS and CV measurements. **Figure 1e** shows EIS of the bare GC, 4-ABA/GC, and Cyt c/4-ABA/GC electrodes. The profiles exhibit a semicircular part followed by a linear part. Compared to the bare GC electrode, the electrode modified with 4-ABA showed a larger charge-transfer resistance. The results indicate that 4-ABA plays an important role in making the electron transfer of $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ more difficult due to the negatively charged moieties at neutral pH. However, the charge-transfer resistance decreased significantly after 4-ABA was covalently linked with Cyt c on the electrode surface, demonstrating the introduction of positively charged Cyt c on the surface. **Figure 1f** shows the CVs of three electrodes in the presence of $Fe(CN)_6^{3-}$. It can be seen that the electron transfer of $Fe(CN)_6^{3-}$ is completely blocked on the 4-ABA/GC electrode due to the negative charges on the surface, and the electron transfer recovered after Cyt c attachment. **Figure 2b** shows the CV of the Cyt c-modified electrode in PBS. A pair of redox peaks was clearly exhibited on the CV with a formal potential at −0.15 V (vs. Ag/AgCl). The density of the electrochemically active pure Cyt c was calculated to be 2.8×10^{-10} mol/cm^2 on the graphite plate surface.

2.2 *In vitro* and *in vivo* electrocatalytic characteristics of Cyt c in the presence of HA

To probe the role of HA in the electron transfer of Cyt c, we studied the electrocatalytic characteristics of Cyt c in the presence of HA using oxygen and sodium thiosulfate as the chemical markers **(Fig. 3a)**. As shown in **Fig. 3b**, HA significantly stimulated the reduction reaction of oxygen on the pure Cyt c-immobilized electrode, indicating the accelerated electron transfer from the electrode toward Cyt c. Meanwhile, HA also stimulated the oxidation of sodium thiosulfate as shown in **Fig. 3c**, suggesting the accelerated electron transfer from Cyt c toward the electrode. Similarly, the electrocatalytic activity of *in vivo* Cyt c increased in the presence of HA. As shown in **Fig. 3d**, the oxygen reduction catalytic behavior of the whole microbial cells was displayed in the presence of HA. A variety of bacteria including both Gram- negative and positive strains have been shown to be able to catalyze oxygen reduction (Cournet et al., 2010). According to a previous study, self-produced mediators and out membrane cytochromes might be responsible for the electron transfer during the microbial-catalyzed oxygen reduction reaction (Freguia et al., 2010). In this case, the oxygen reduction reaction was conducted at the no-turnover state with fresh PBS solution as the electrolyte, which excluded the effect of self-produced flavins on the reaction. Instead, HA was applied in the system, which might have similar functions as the flavins to stimulate the electron transfer of *in vivo* Cyt c for the oxygen reduction reaction. As a result of the presence of HA, the oxygen reduction catalytic current of

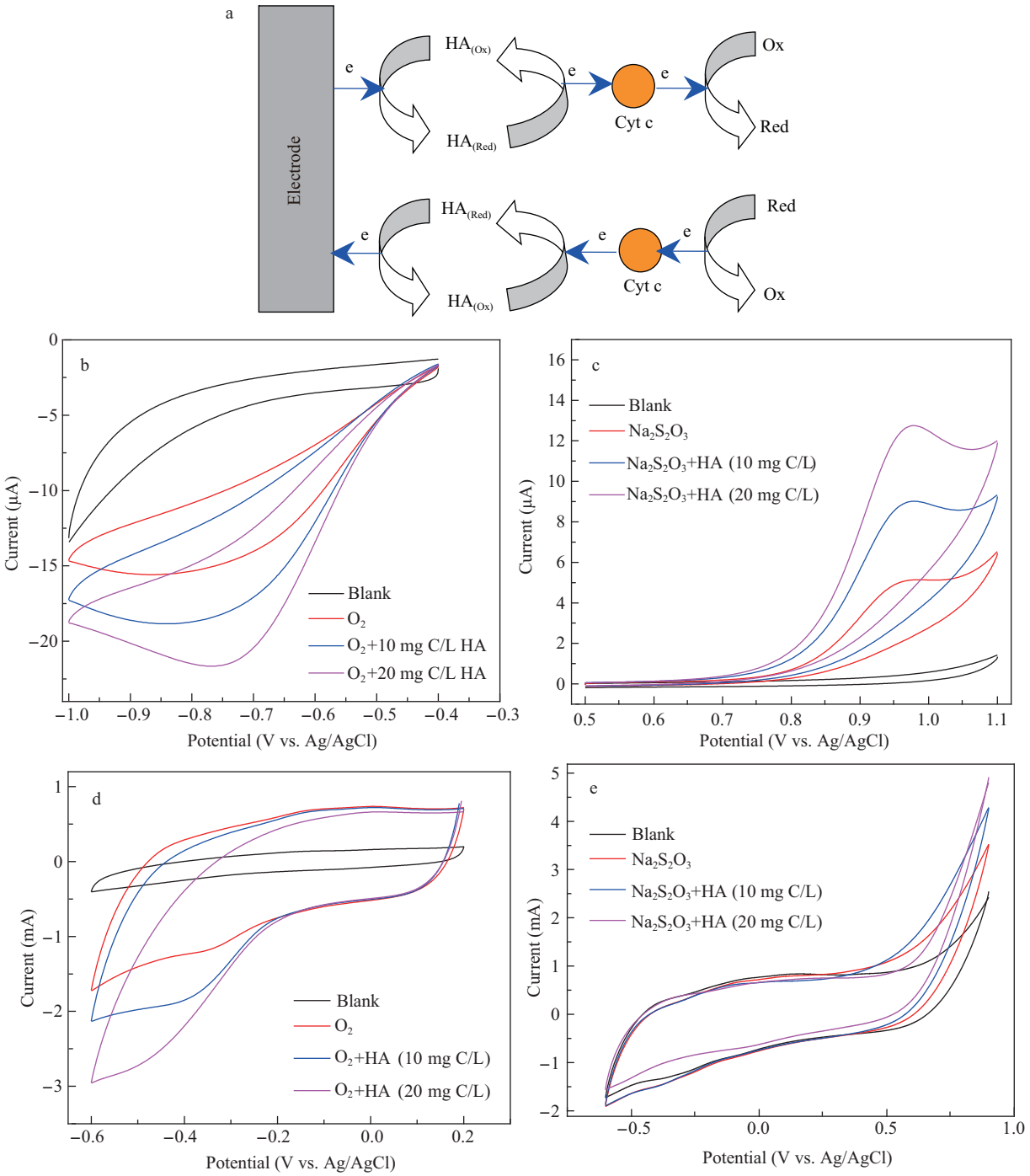


Fig. 3 Scheme of the HA mediated electron transfer of Cyt c in the presence of oxygen or $Na_2S_2O_3$ (a), CV scans of Cyt c/4-ABA/GC electrode in the presence of oxygen (b), $Na_2S_2O_3$ (c), and HA; CV scans of MR-1 attached electrode in the presence of oxygen (d) and $Na_2S_2O_3$ (e), and HA.

the whole cells increased about 60% as compared to that in the absence of HA. HA also stimulated the oxidation of sodium thiosulfate as shown in **Fig. 3d**, suggesting the accelerated electron transfer from *in vivo* Cyt c toward the electrode. All the results indicated that HA might act as electron shuttles, facilitating the electron transfer between the electrode and Cyt c, which can enhance the electrochemical activity of both *in vitro* and *in vivo* Cyt c.

2.3 *In vitro* and *in vivo* spectroscopic characteristics of Cyt c in the presence of HA

The stimulated electron transfer of Cyt c by HA was believed to be due to the molecular interaction between the negatively charged HA and positively charged Cyt c. **Figure 4a** shows the UV-vis spectra of *in vivo* Cyt c in the presence of HA. MR-1 with OM-bonded Cyt c showed distinctive UV-Vis spectra. Oxidized Cyt c has a soret

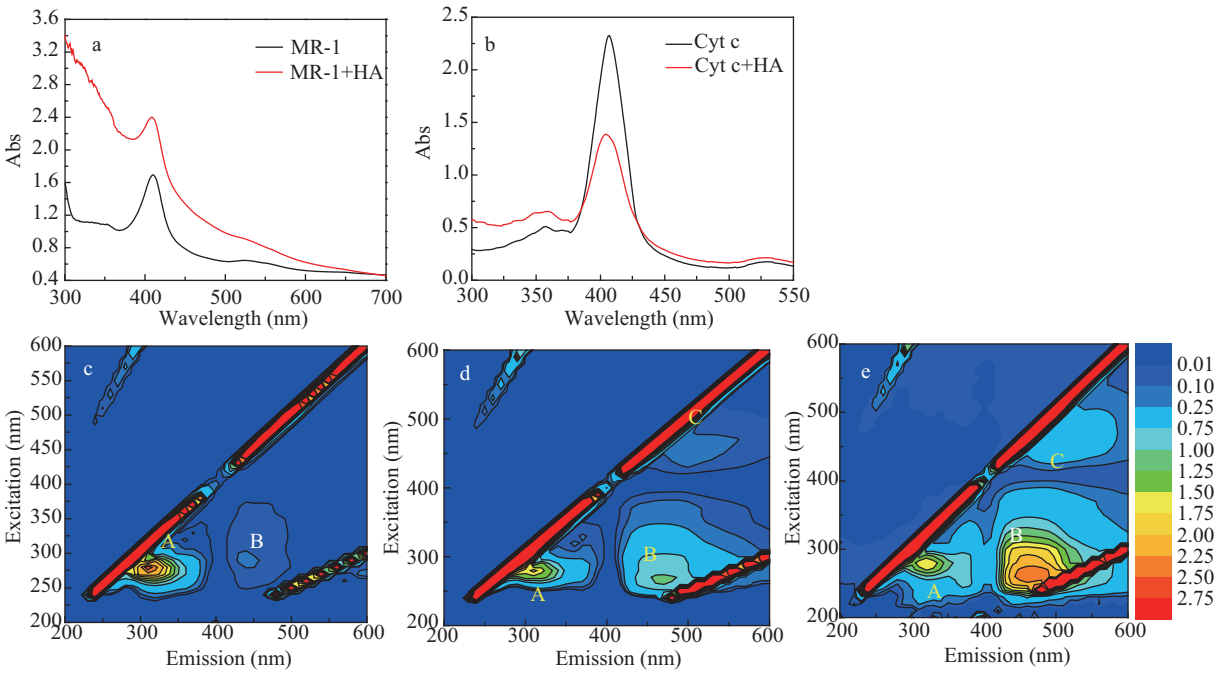


Fig. 4 UV-Vis spectra of the mixture of MR-1 and HA (a) and the mixture of the horse-heart Cyt c and HA (b). The EEM spectra of the horse-heart Cyt c (c), HA (d) and the mixture of Cyt c and HA (e).

band at 409.5 nm and a broad band at 528 nm. These spectral features are typical of hexacoordinated low-spin hemes (Collinson et al., 1992). It is noted that the blue-shifting of the soret band of *in vivo* Cyt c from 409.5 to 408 nm was observed in the presence of HA, suggesting possible interaction between Cyt c and HA. Similarly, the soret band of the pure Cyt c shifted from 406 to 404 nm in the presence of HA (**Fig. 4b**), further confirming the interaction between HA and Cyt c. Meanwhile, the suggested molecular interaction was also confirmed by EEM. As shown in **Fig. 4c**, the EEM fluorescence spectrum of Cyt c was characterized by two main fluorophores corresponding to peak A and peak B. Peak A was located at Ex/Em = 310/280 nm with a relatively high fluorescence intensity (FI) value of 2.72, and peak B was centered at Ex/Em = 440/290 nm with an FI value of 0.12. The EEM fluorescence spectrum of HA was characterized by three fluorophores corresponding to peak A, peak B, and peak C (**Fig. 4d**). Peak A also was located at Ex/Em = 310/280 nm with an FI value of 1.87, peak B at Ex/Em = 466/270 nm with an FI value of 1.05, and peak C at Ex/Em = 500/450 nm with an FI value of 0.16. The complex of Cyt c and HA showed a different fluorescence spectrum (**Fig. 4e**). Similar to that of HA, the fluorescence spectrum of the complex was characterized by the three fluorophores of peak A, peak B, and peak C, but the peak positions as well as the FI values were different. Peak A was located at Ex/Em = 320/280 nm with an FI value of 1.73, peak B at Ex/Em = 460/260, and peak C at Ex/Em = 500/449 with an FI value of 0.52. It is well known that Cyt c has nine positive charges at pH 7.0, resulting from the protonated amino groups (Armstrong et al., 1988). HA is rich in basic units, involving a proportion of more or less condensed aromatic rings connected with phenol groups and a large number of attached chelant groups such as –COOH (Luisella et al., 1997), which are capable of interacting with the amino groups to form organic complexes through electrostatic or hydrogen-bonding interactions, such as $-NH_3^+ \cdots$–OOC–R or N–H$\cdots$ O–C_6H_4–R (Xu et al., 2004).

3 Conclusions

In summary, OM Cyt c, as an extracytoplasmic electron sink in microbes, plays a very important role in EET. However, the low electron transfer rate of the Cyt c greatly hinders the whole EET process and further limits the practical application of this technology. Addition of exogenous electron shuttles was proved to be able to improve EET efficiency. Reported electron mediators are mainly compounds containing quinone groups, such as anthraquinone-2,6-disulfonate, thionin, neutral red, 2-hydroxy-1,4-naphtoquinone, and benzoquinone. However, many of these compounds are toxic and costly. HA are ubiquitous in natural environments, and can be a potential alternative to these chemical mediators. However, the inherent mechanism of the HA-stimulated EET has not been well interpreted so far. This study focused on probing the mechanism behind HA stimulation of EET by revealing the interaction between HA and Cyt c. Electrochemical approaches found that HA could facilitate the

electron transfer of both *in vivo* and *in vitro* Cyt c with oxygen and sodium thiosulfate as chemical markers. *In vivo* and *in vitro* spectroscopic analyses demonstrated that the amino groups of Cyt c interacted with HA to form organic complexes via electrostatic or hydrogen-bonding interactions. The results suggested that the stimulation of EET by HA likely resulted from the interaction between Cyt c and HA, by which HA facilitated electron transfer from Cyt c towards solid electron acceptors. These findings help in understanding the biogeochemical role of humic substances in the environment.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 21277035, 21177030), the Natural Science Foundation of Guangdong Province, China (No. S2012030006144), and the Foundation for Excellent Young Scientist in Guangdong Academy of Sciences (No. rcjj201101).

REFERENCES

Armstrong, F. A., Allen, H., Hill, O., Walton, N. J., 1988. Direct electrochemistry of redox proteins. Acc. Chem. Res. 21(11), 407–413.

Baron, D., Labelle, E., Coursolle, D., Gralnick, J. A., Bond, D., 2009. Electrochemical measurement of electron transfer kinetics by *Shewanella oneidensis* MR-1. J. Biol. Chem. 284(42), 28865–28873.

Bond, D. R., Holmes, D. E., Tender, L. M., Lovley, D. R., 2002. Electrode-reducing microorganisms that harvest energy from marine sediments. Science 295(5554), 483–485.

Collinson, M., Bowden, E. F., Tarlov, M. J., 1992. Voltammetry of covalently immobilized cytochrome c on self-assembled monolayer electrodes. Langmuir 8(5), 1247–1250.

Cournet, A., Délia, M., Bergel, A., Roques, C., Bergé, M., 2010. Electrochemical reduction of oxygen catalyzed by a wide range of bacteria including Gram-positive. Electrochem. Commun. 12(4), 505–508.

Esteve-Núñez, A., Sosnik, J., Visconti, P., Lovley, D. R., 2008. Fluorescent properties of c-type cytochromes reveal their potential role as an extracytoplasmic electron sink in *Geobacter sulfurreducens*. Environ. Microbiol. 10(2), 497–505.

Freguia, S., Tsujimura, S., Kano, K., 2010. Electron transfer pathways in microbial oxygen biocathodes. Electrochim. Acta 55(3), 813–818.

Jiang, J., Kappler, A., 2008. Kinetics of microbial and chemical reduction of humic substances: implications for electron shuttling. Environ. Sci. Technol. 42(10), 3563–3569.

Keller, J. K., Weisenhorn, P. B., Megonigal, J. P., 2009. Humic acids as electron acceptors in wetland decomposition. Soil Biol. Biochem. 41(7), 1518–1522.

Kim, H. J., Park, H. S., Hyun, M. S., Chang, I. S., Kim, M., Kim, B. H., 2002. A mediator-less microbial fuel cell using a metal reducing bacterium, *Shewanella putrefaciens*. Enzyme Microb. Technol. 30(2), 145–152.

Lovely, D. R., Coates, J. D., Bluntharris, E. L., Phillips, E. P., Woodward, J. C., 1996. Humic substances as electron acceptors for microbial respiration. Nature 382(6590), 445–448.

Lovley, D. R., Blunt-Harris, E. L., 1999. Role of humic-bound iron as an electron transfer agent in dissimilatory Fe(III) reduction. Appl. Environ. Microbiol. 65(9), 4252–4254.

Luisella, C., Morris, S., Michèle, N., 1997. Analysis of carboxyl groups in soil humic acids by a wet chemical method, fourier-transform infrared spectrophotometry, and solution-state carbon-13 nuclear magnetic resonance: a comparative Study. Soil Sci. 162(3), 189–197.

Nielsen, L. P., Risgaard-Petersen, N., Fossing, H., Christensen, P. B., Sayama, M., 2010. Electric currents couple spatially separated biogeochemical processes in marine sediment. Nature 464(7284), 1071–1074.

Peng, L., You, S. J., Wang, J. Y., 2010. Electrode potential regulates cytochrome accumulation on *Shewanella oneidensis* cell surface and the consequence to bioelectrocatalytic current generation. Biosens. Bioelectron., 25(11), 2530–2533.

Peretyazhko, T., Sposito, G., 2005. Iron(III) reduction and phosphorous solubilization in humid tropical forest soils. Geochim. Cosmochim. Acta 69(14), 3643–3652.

Rabaey, K., Boon, N., Höfte, M., Verstraete, W., 2005. Microbial phenazine production enhances electron transfer in biofuel cells. Environ. Sci. Technol. 39(9), 3401–3408.

Reguera, G., McCarthy, K. D., Mehta, T., Nicoll, J. S., Tuominen, M. T., Lovley, D. R., 2005. Extracellular electron transfer via microbial nanowires. Nature 435(7045), 1098–1101.

Roden, E. E., Kappler, A., Bauer, I., Jiang, J., Paul, A., Stoesser R. et al., 2010. Extracellular electron transfer through microbial reduction of solid-phase humic substances. Nat. Geosci. 3(6), 417–421.

Scott, D. T., McKnight, D. M., Blunt-Harris, E. L., Kolesar, S. E., Lovley, D. R., 1998. Quinone moieties act as electron acceptors in the reduction of humic substances by humics-reducing microorganisms. Environ. Sci. Technol. 32(19), 2984–2989.

Shi, L., Richardson, D. J., Wang, Z. M., Kerisit, S. N., Rosso K. M. et al., 2009. The roles of outer membrane cytochromes of *Shewanella* and *Geobacter* in extracellular electron transfer. Environ. Microbiol. Rep. 1(4), 220–227.

Videla, H. A., Herrera, L. K., 2009. Understanding microbial inhibition of corrosion: A comprehensive overview. Int. Biodeterior. Biodegrad. 63(7), 896–900.

Wolf, M., Kappler, A., Jiang, J., Meckenstock, R. U., 2009. Effects of humic substances and quinones at low concentrations on ferrihydrite reduction by *Geobacter metallireducens*. Environ. Sci. Technol. 43(15), 5679–5685.

Xu, J. J., Wang, G., Zhang, Q., Zhou, D. M., Chen, H. Y., 2004. Interfacing cytochrome c to Au electrodes with humic acid film. Electrochem. Commun. 6(3), 278–283.

Yang, G. C., Shen, Y., Wang, M., Chen, H., Liu, B., Dong, S., 2006. Copper hexacyanoferrate multilayer films on glassy carbon electrode modified with 4-aminobenzoic acid in aqueous solution. Talanta 68(3), 741–747.

Yuan, Y., Zhou, S. G., Zhuang, L., 2010. A new approach to in situ sediment remediation based on air-cathode microbial fuel cells. J. Soils Sedim. 10(7), 1427–1433.