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A role for microbial–palladium nanoparticles in extracellular electron transfer**

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Insights into extracellular electron transfer of microorganisms are important for the understanding of electron transport processes in bioelectrochemical systems (e.g. biological fuel cells and microbial electrolysis cells), as well as for biogeochemical cycles, biocorrosion and bioremediation[1-7]. Two principal mechanisms for extracellular electron transfer have been proposed: i) electroactive metabolites/secretions serve as mediators in an indirect electron transfer process; ii) electrons transfer directly from the cells to the electrodes via either membrane cytochromes or electrically conductive pili – the latter process has been defined in a termed direct electron transfer (DET) and has recently been the subject of intense study[3–7]. It is well known that several microbes are capable of transforming a range of metal ions/minerals into nanoparticles that are bound into the cell membrane. However, little is understood about the role of such metallic nanoparticles in the physiological electron transfer processes, and important questions remain regarding the detailed mechanisms involved.

In this work, the sulfate–reducing bacterium *D. desulfuricans* strain was chosen as a model organism to study the direct electrochemistry of microbes and the role of transformed metals. Native cells appeared as thin rods with a homogenous surface (Figure 1). After exposing these cells to Pd(II) cations in an aqueous lactate solution, the bacterial surface presented dense dark spots in the images; these exhibited a range of shapes and had an average diameter of less than 10 nm and were confirmed as Pd metal nanoparticles by EDX analysis.

DET of native cell. Direct evidence from the washed cells was obtained using cyclic voltammetry. Curve 2 in Figure 2a shows oxidation peaks at –0.39 and 0.05 V and reduction peaks at –0.45 and –0.26 V in 50 mM buffer. An increase of pH caused a negative shift of potential in the oxidative/reductive waves, with a slope of –60 mV pH^{-1}, suggesting that proton concentration gradients are involved in the extracellular electron transfer process. The experiments were conducted under conditions that excluded the possibility of indirect electron transfer reactions; hence, the redox waves are due to the DET of enzymes bound on the membranes of the cells. The results corroborate the assumption that the direct mechanism (i.e. DET) is preferred by sulfate–reducing bacteria when obtaining electrons from metals (as in the corrosion of iron), rather than involving intermediate corrosive agents such as H^+ or HS^-[8].

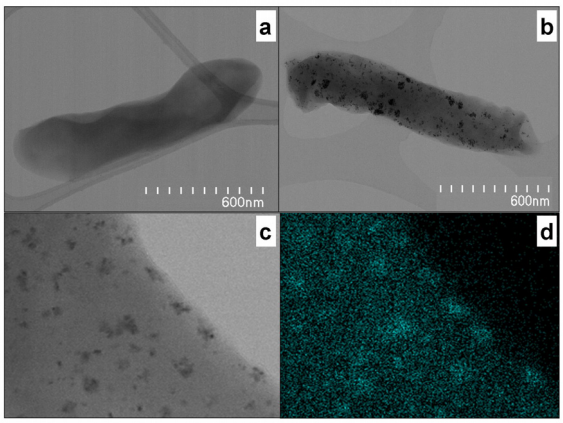

Figure 1. STEM images of: a) a pristine *D. desulfuricans* cell; b) a D. *desulfuricans* cell after exposure to 1 mM Pd(II) solution for 3 h with lactate as electron donor; c) microscopic detail of a washed Pd–loaded cell (magnification × 300000); and d) localization of bio–deposited Pd(0) particles examined by EDX mapping (magnification × 300000).

The open circuit potential of *D. desulfuricans* modified glassy carbon is approximately –0.23 V, and originates from the electrode reactions of membrane enzymes linking oxidative metabolism to extracellular electron transfer; this value would undergo a negative shift in potential in the presence of microbially produced sulfide[9,10]. Microbes obtain electrons from substrates which are oxidized through metabolic processes. A comparison of curves 2 and 3 in Figure 2a reveals that the oxidation peak at 0.1 V is linked to the electron pathway of lactate oxidation due to an increase in catalytic current. The formal potential of lactate oxidation to acetate has been reported to be at –0.42 V[7], however, the catalytic current in Figure 2a can be seen to start at –0.06 V, which is consistent with the reported value for a cytochrome isolated from *Shewanella*[11]. Cytochrome *c3* has been demonstrated to act as an electron carrier for hydrogenase reactions in *D. desulfuricans*, to either utilize or produce hydrogen depending on the growth conditions[12]. In our case, a membrane–bound cytochrome *c3* could act as a conduit for extracellular electron transfer to the electrode, while periplasmic hydrogenases may provide a pathway for electrons transport from the substrate oxidation; in other words, cytochromes and hydrogenases are interacting to enable the transfer of electrons to the electrode. Scheme 1a shows the proposed electron transfer chain based on the data from the literature[12–14]. As lipid membranes are not electronically conductive, the proposed route of electron transfer between proteins in the cell membranes is determined by their location (or

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translocation); the sequential multi–step reactions depend on the relevant energy states (i.e. Gibbs free energy changes for the enzyme catalyzed redox reactions) involved. In *Desulfovibrio species*, substrates provide electrons via oxidation reactions catalyzed by enzymes such as lactate dehydrogenase or formate dehydrogenase. Periplasmic hydrogenases then transport the electrons via reactions involving hydrogen or proton translocation; and some electrons are terminally transported to electrodes *via* cytochromes present in the membrane[12–14].

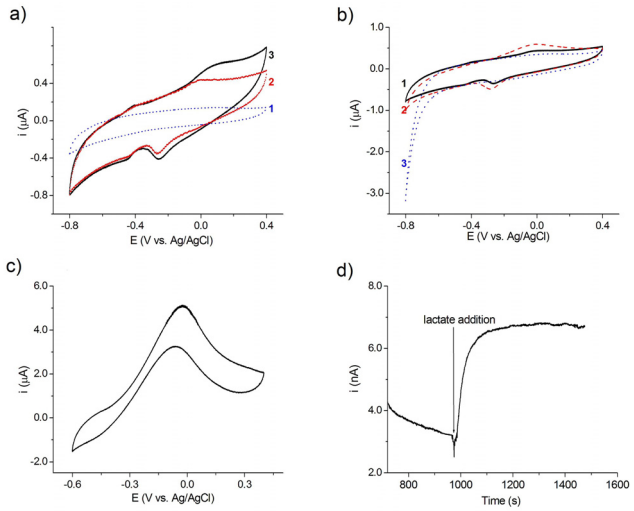
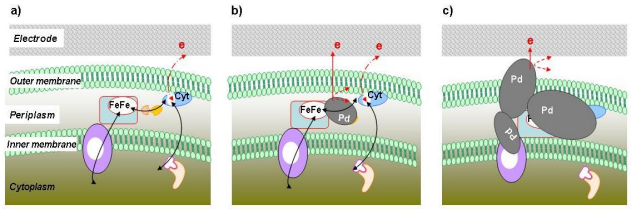


Figure 2. a) Cyclic voltammograms of: (1) bare GC electrode; washed *D. desulfuricans* (Pd-free) cells coated onto a GC electrode in the absence (2) and presence (3) of lactate in 0.05 M buffer under a nitrogen atmosphere. b) Cyclic voltammograms of: (1) *D. desulfuricans* coated onto a GC electrode; (2) *D. desulfuricans* cells exposed to 0.07 mM Pd(II) coated onto a GC electrode; (3) *D. desulfuricans* cells exposed to 1.0 mM Pd(II) coated onto a GC electrode. Scan rate 10 mV/s. c) Cyclic voltammograms in the presence of formate of a GC electrode coated with *D. desulfuricans* cells exposed to 1 mM Pd(II). d). Potentiostatic lactate oxidation with a working electrode consisting of Pd(0)-*D. desulfuricans* coated onto GC and held at a potential of 0.20 V vs. Ag/AgCl. 0.2 M lactate was added in the buffer at t = 960 s.

DET of low-load-Pd cells. Microorganisms are capable of transforming metal ions into metal (e.g. palladium, platinum and gold) nanoparticles[15–18]; these elements, like enzymes, have outstanding catalytic properties in a wide range of redox reactions. Moreover, the metal particles have a higher electronic conductivity compared to the microbial pili (~0.1 S/cm)[5] of extracellular electron transfer. To investigate the role of the nanoparticles in the physicochemical reactions, the microbes were first exposed to Pd(II) in concentrations of 0.07 and 1 mM to obtain different loadings[19] of cell bound Pd(0) particles; these cells were then coated onto GC electrodes for electrochemical testing. Compared to the benchmark Pd–free *D. desulfuricans* modified GC experiment (curve 1 in Figure 2b), the 0.07 mM-Pd(II)-exposed cells (curve 2 in Figure 2b) yielded higher lactate oxidation currents in the range –0.4 to 0.2 V which shows that the electron transport increased with the presence of bio–derived Pd(0). It has been known that environmental conditions significantly influence the physiological state of microbes and can even impact upon the substrates or enzymes that can be utilized as temporary electron acceptors[20], and electrons from lactate oxidation are capable of transport through two or more pathways[13]. In the present case, the periplasmic cytochrome *c3* and hydrogenases are involved in the electron transfer processes for lactate oxidation and for initial Pd(II) ions reduction[21] and hydrogenases act as the nucleation sites of phase transformation[17]. When a low amount of Pd(0) is present alongside the periplasmic hydrogenases and cytochromes, the metallic particles are hypothesised to facilitate electron transfer between the cells and electrode due to the higher conductivities and lower energy states compared to the proteins (illustrated in Scheme 1b). Curve 2 in Figure 2b shows large reduction currents at low potentials in the region corresponding to proton reduction (i.e. H_2 evolution); this data suggests that the microbial–derived Pd particles exhibit a high catalytic activity for the production of molecular hydrogen, which is a key intermediate in the energy metabolism of a wide range of microbes. The increased current of lactate oxidation and of hydrogen evolution raises a possibility that the Pd nanoparticles participate in microbial metabolism as a bio–derived electron carrier, a mechanism which may be further confirmed by molecular biology or combined electrochemistry-spectroscopy techniques[6].



Scheme 1. Hypothesised pathways for extracellular electron transfer chain between cell and electrode: a) via periplasmic cytochromes and hydrogenases (absence of bio–Pd); b) presence of low loaded bio–Pd(0); and c) presence of high loaded bio–Pd(0). [FeFe] represents iron–only hydrogenases, Cyt represents c–type cytochromes and grey shaded areas the bio–Pd(0) nanoparticles.

DET of high-load-Pd cells. The tolerance of microbes towards metal ions and the growth of the elemental particles depend on the physicochemical parameters[22]. Compared to the benchmark Pd–free *D. desulfuricans* modified GC, cells exposed to the 1 mM Pd(II) (curve 3 in Figure 2a) exhibited no obvious redox peaks in the range of –0.4 to 0.2 V but there was a positive shift in the open circuit potential, indicating an influence from the interaction of the enzymes and Pd(0) particles. The electrode reaction for this high loaded Pd system is illustrated in Scheme 1c. When the Pd metal particles grow in size and spatially dominate around the positions of periplasmic hydrogenases and cytochromes, they will interfere with the natural enzymatic electron transfer pathways and catalytic reactions due to their high electronic conductive capacity. In Figure 2c, an oxidation current of 5.1 μA (forward scan) was achieved with the presence of formate instead of lactate. Upon reversing the potential sweep direction, an oxidation current of 3.2 μA was obtained. Considering the observed high currents and the profile of the voltammogram, abiotic direct electrochemical oxidation of formate on the surfaces of the bio–Pd(0) electrocatalysts is probably due to the intrinsic catalytic activity of the membrane–bound particles.

When potentiostatic investigations were undertaken, a stable lactate oxidation current was obtained (Figure 2d); the changes in current on addition of electron donors for a variety of prepared electrodes are shown in Table 1. For the control experiments, no change in oxidation current was detected on addition of lactate. The low loaded Pd–*D. desulfuricans*–GC yielded a stable current of 25 nA, a factor of 4 greater compared to the Pd–free *D. desulfuricans*–GC control; this indicates that the Pd nanoparticles

bound onto the cell membranes facilitate the electron transfer. No lactate oxidation currents were detected for the high loaded Pd–*D. desulfuricans*–GC electrode, which indicates that the Pd(0) interferes with the microbial enzyme reactions in the cell membrane at these loadings. However, the particles can act as highly active bio–nanocatalysts for electrode reactions in presence of formate and hydrogen etc. and this could have potential environmental applications in bio-remediation processes[19].

Table 1. Summary of potentiostatic data for substrate oxidation on various working electrodes poised at +0.20 V vs. Ag/AgCl.

Electrode type	Substrate	Current increase (nA) on substrate addition
Pristine GC	Lactate	n.d.
Pd–only modified GC	Lactate	n.d.
D. desulfuricans–GC[b]	Lactate	n.d.
D. desulfuricans–GC[a]	Lactate	6.1 ± 5.3
D. desulfuricans–Pd(0)–GC (low Pd loading)[a]	Lactate	25 ± 17
D. desulfuricans–Pd(0)–GC (low Pd loading)[b]	Lactate	n.d.
D. desulfuricans–Pd(0)–GC (high Pd loading)[a]	Lactate	n.d.
Pd–only modified GC	Formate	191 ± 87
D. desulfuricans–Pd(0)–GC (high Pd loading)[a]	Formate	232 ± 65

Note: [a]electrode was prepared under nitrogen condition; [b]electrode was prepared as a control experiments *via* an air–dried procedure to obtain dead Pd-cells. n.d. = none detected. Data presented with standard deviations (n = 6).

Herein we have demonstrated a DET mechanism used by *D. desulfuricans*; where the periplasmic cytochromes and hydrogenases play an important role, and Pd nanoparticles bound to the microbes may participate in the electron transfer process. The present work is of importance not only for the fundamental studies of electron transfer processes in microbial physiology and ecology, but also for increased understanding and improvement of the performance of bioelectrochemical techniques *e.g.* precious metals are extensively used and important catalysts, and therefore present in many industry processing wastewaters. Bio-nanoparticles can oxidize in situ metabolites *e.g.* H_2, formate and ethanol in the anode chambers, while also acting as cathodic catalysts for the oxygen reduction reaction[23]. In addition, this study indicates the feasibility of using bioelectrochemical systems for metal immobilization, recovery or detoxification.

Experimental Section

Cultivation conditions: D. desulfuricans strain was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). The cells were grown in sealed bottles for 3 days at 30°C in the medium previously described[9,10], collected by centrifugation at 3500 rpm for 15 min. Pellets were washed two times with 50 mL of 20 mM morpholinepropanesulfonic acid (MOPS)–NaOH buffer (pH = 6.9) to remove any residual sulfide required prior to bio–Pd(0) preparation. All solutions were purged with nitrogen before use and anaerobic conditions were maintained throughout the study unless otherwise stated.

Pd(II) reduction by D. desulfuricans: Microbial pellets were added to 10 mL sterile MOPS buffer with 5 mM lactate as electron donor. A concentrated stock solution of ammonium tetrachloropalladate(II) was added to a yield a final concentration of 0.07 and 1.0 mM respectively. The cultures were maintained different time (0, 1, 2, 3, 4 and 24 h) at 30 ± 1 or 22 ± 1 °C for the Pd(II) reduction process.

Microbially modified electrode preparation: After the reduction, the cells were harvested by centrifugation at 3500 rpm for 15 min, further washed with MOPS buffer and water (> 18.2 M/cm), anaerobically transferred onto a the surface of a polished glassy carbon electrode (GC, 3 mm diameter), and dried at room temperature under a nitrogen atmosphere (in a control experiment, to show that the Pd-bacteria produced the electric current, the bacteria were killed by either exposing them to air (these bacteria are anaerobes) or by heat treatement (details?)).

Electrochemical measurements: The electrochemical characterization was carried out by cyclic voltammetry and potentiostatic measurements using a computer–controlled Autolab potentiostat/galvanostat with a three–electrode chamber containing a platinum wire counter electrode and a Ag/AgCl reference electrode. All potentials in this paper refer to this reference electrode. 0.05 M buffer (no culture medium) was used as electrolyte.

Electron microscopy and energy–dispersive X–ray: Cells suspended in the MOPS buffer were fixed for 1 h in 2.5%(w/v) glutaraldehyde, and then dehydrated by using an ethanol in water series (25, 50, 75 and 100%), in steps was of 15 min duration. The suspension was air dried for 12 h and then placed onto a carbon–coated copper grid for electron microscopy analysis. A Hitachi HD2300A scanning transmission electron microscope with an EDX detector was used to characterize the cells and the Pd(0) particles with an acceleration voltage of 200 kV.

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