

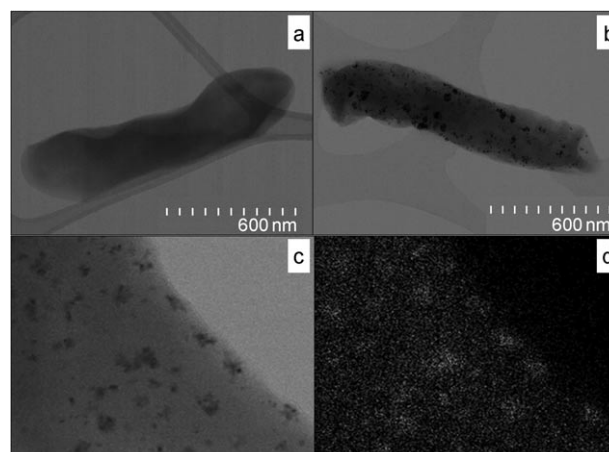
# A Role for Microbial Palladium Nanoparticles in Extracellular Electron Transfer\*\*

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Insight into extracellular electron transfer of microorganisms is important for our understanding of electron-transport pathways in bioelectrochemical systems (e.g. biological fuel cells and microbial electrolysis cells), as well as for biogeochemical cycles, biocorrosion, and bioremediation.<sup>[1–7]</sup> Two principal mechanisms for extracellular electron transfer have been proposed: 1) electroactive metabolites/secretions serve as mediators in an indirect electron-transfer process; 2) electrons transfer directly from the cells to the electrodes via either membrane cytochromes or electrically conductive pili. The latter process has been defined as direct electron transfer (DET) and has been studied intensely.<sup>[3–7]</sup> It is well known that several microbes are capable of transforming a range of metal ions/minerals into nanoparticles, which remain bound to the cell membrane. However, little is understood about the role of such metallic nanoparticles in physiological electron-transfer processes, and important questions remain regarding the details of the mechanisms involved.

In this study, we chose the sulfate-reducing bacterium *Desulfovibrio desulfuricans* 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 the exposure of these cells to Pd<sup>II</sup> cations in an aqueous lactate solution, images of the bacterial surface showed dense dark spots with a range of shapes and an average diameter of less than 10 nm. Analysis by energy-dispersive X-ray spectroscopy (EDX) confirmed that the spots were palladium(0) nanoparticles.

Direct evidence from the washed cells was obtained by cyclic voltammetry. Oxidation peaks were observed at –0.39 and 0.05 V and reduction peaks at –0.45 and –0.26 V in 50 mM buffer (Figure 2a, curve 2). An increase in the



**Figure 1.** a) STEM image of a pristine *D. desulfuricans* cell. b) STEM image of a *D. desulfuricans* cell after exposure to a solution of Pd<sup>II</sup> (1 mM) for 3 h with lactate as the electron donor. c) STEM image showing the microscopic detail of a washed palladium-loaded cell. d) Localization of the biodeposited Pd<sup>0</sup> particles by EDX mapping (magnification  $\times 300000$ ).

pH value caused a negative shift in potential in the oxidative/reductive waves, with a slope of –60 mV/pH. This result suggests 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 were due to the DET of enzymes bound to the membranes of the cells. The results corroborate the assumption that the direct mechanism (i.e. DET) is preferred by sulfate-reducing bacteria when they obtain electrons from metals (as in the corrosion of iron).<sup>[8]</sup>

The open-circuit potential of glassy carbon (GC) modified with *D. desulfuricans* 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.<sup>[9,10]</sup> Microbes obtain electrons from substrates that 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: there is an increase in catalytic current in the presence of lactate. The formal potential of lactate oxidation to acetate has been reported to be –0.42 V;<sup>[7]</sup> 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*.<sup>[11]</sup> Cytochrome *c*<sub>3</sub> has been demonstrated to act as an electron

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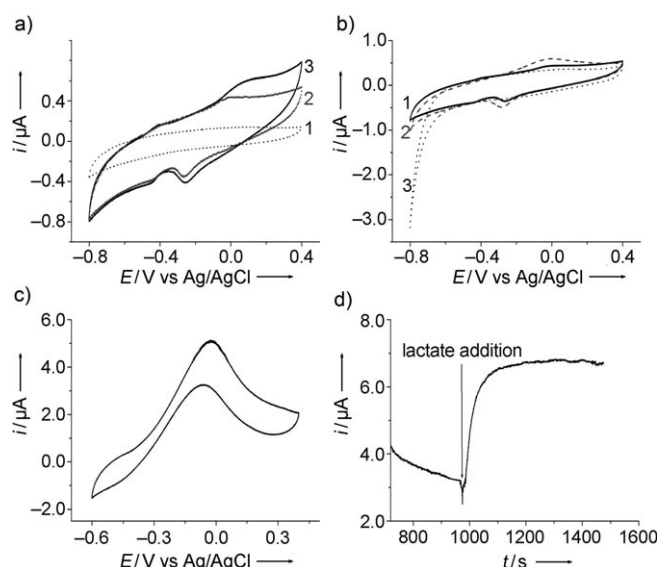
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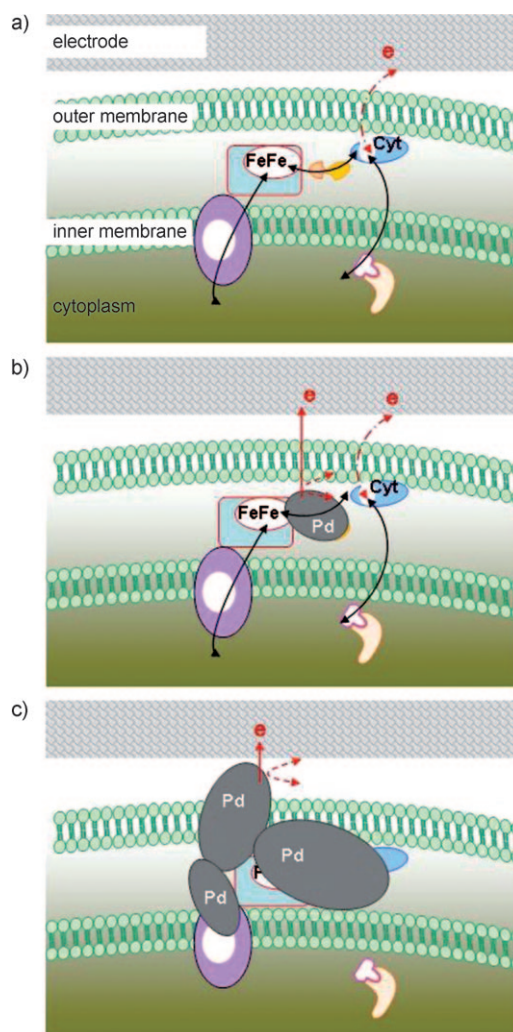


**Figure 2.** a) Cyclic voltammograms of: 1) the bare GC electrode; 2,3) washed *D. desulfuricans* (palladium-free) cells coated on a GC electrode in the absence (2) and presence (3) of lactate in a buffer (0.05 M) under a nitrogen atmosphere. b) Cyclic voltammograms of: 1) *D. desulfuricans* coated on a GC electrode; 2) cells coated on a GC electrode with a low palladium loading; 3) cells coated on a GC electrode with a high palladium loading. Scan rate:  $10 \text{ mV s}^{-1}$  for all experiments. c) Cyclic voltammogram of cells coated on a GC electrode with a high palladium loading in the presence of formate. d) Potentiostatic lactate oxidation with a working electrode consisting of  $\text{Pd}^0$ -*D. desulfuricans* coated on GC and held at a potential of 0.20 V versus Ag/AgCl. Lactate (0.2 M) was added to the buffer at  $t = 960 \text{ s}$ .

carrier for hydrogenase reactions in *D. desulfuricans*. It either utilizes or produces hydrogen, depending on the growth conditions.<sup>[12]</sup> In our case, membrane-bound cytochrome  $c_3$  could act as a conduit for extracellular electron transfer to the electrode, whereas periplasmic hydrogenases may provide a pathway for electron transport from the substrate during oxidation. In other words, cytochromes and hydrogenases interact to enable the transfer of electrons to the electrode.

Figure 3a shows the proposed electron-transfer chain based on reported data.<sup>[12–14]</sup> 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 translocation); the sequential multistep reactions depend on the relevant energy states involved (an example: changes in Gibbs free energy for enzyme-catalyzed redox reactions). In *Desulfovibrio species*, substrates provide electrons through oxidation reactions catalyzed by enzymes such as lactate dehydrogenase or formate dehydrogenase. Periplasmic hydrogenases then transport the electrons through reactions involving hydrogen or proton translocation; and some electrons are terminally transported to electrodes via cytochromes present in the membrane.<sup>[12–14]</sup>

Microorganisms are capable of transforming metal ions into metal (e.g. palladium, platinum, gold) nanoparticles.<sup>[15–19]</sup> These elements, like enzymes, have outstanding catalytic properties in a wide range of redox reactions. Moreover, the



**Figure 3.** Hypothesized pathways for the extracellular electron-transfer chain between the cell and the electrode: a) in the absence of  $\text{Pd}^0$  (via periplasmic cytochromes and hydrogenases); b) in the presence of  $\text{Pd}^0$  at a low loading; c) in the presence of  $\text{Pd}^0$  at a high loading. [FeFe] is an iron-only hydrogenase, Cyt is a  $c$ -type cytochrome, and areas shaded gray are the  $\text{Pd}^0$  nanoparticles.

metals have a higher electronic conductivity than the microbial pili (ca.  $0.1 \text{ Scm}^{-1}$ )<sup>[5]</sup> for extracellular electron transfer. To investigate the role of the nanoparticles in the physicochemical reactions, we first exposed the microbes to  $\text{Pd}^{\text{II}}$  in concentrations of 0.07 and 1 mM to create cell-bound  $\text{Pd}^0$  particles at different loadings.<sup>[19]</sup> The cells were then coated onto GC electrodes for electrochemical testing. Higher lactate-oxidation currents were observed in the range  $-0.4$ – $0.2 \text{ V}$  for the cells exposed to 0.07 mM  $\text{Pd}^{\text{II}}$  (curve 2 in Figure 2b) than in the benchmark experiment in which GC was modified with palladium-free *D. desulfuricans* (curve 1 in Figure 2b). Thus, electron transport increased in the presence of biologically derived  $\text{Pd}^0$ .

In the present case, the periplasmic cytochrome  $c_3$  and hydrogenases are involved in the electron-transfer processes for lactate oxidation and for the initial reduction of  $\text{Pd}^{\text{II}}$  ions,<sup>[20]</sup> and hydrogenases act as nucleation sites for phase transformation.<sup>[17]</sup> Environmental conditions significantly

influence the physiological state of microbes and even impact upon the substrates or enzymes that can be utilized as temporary electron acceptors,<sup>[21]</sup> and electrons released by lactate oxidation can be transported through two or more pathways.<sup>[13]</sup> We hypothesize that when a low amount of Pd<sup>0</sup> is present alongside the periplasmic hydrogenases and cytochromes, the metallic particles facilitate electron transfer between the cells and the electrode as a result of their higher conductivity and lower energy relative to those of the proteins (Figure 3b). The large reduction currents observed at low potentials in the region corresponding to proton reduction (i.e. H<sub>2</sub> evolution; curve 2 in Figure 2b) suggest that the Pd particles derived from interaction with the microbe exhibit 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 corresponding to lactate oxidation and hydrogen evolution raises the possibility that the nanoparticles participate in metabolism as biologically derived electron carriers: a mechanism which may be further confirmed by molecular-biology or combined electrochemical-spectroscopic techniques.<sup>[6]</sup>

The tolerance of microbes towards metal ions and the growth of the elemental particles depends on the physicochemical parameters.<sup>[22]</sup> In comparison to the benchmark GC modified with palladium-free *D. desulfuricans*, cells exposed to 1 mM Pd<sup>II</sup> (curve 3 in Figure 2a) exhibited no evident redox peaks in the range -0.4–0.2 V; however, there was a positive shift in the open-circuit potential. This shift indicated an influence from the interaction of the enzymes with the Pd<sup>0</sup> particles. The probable electrode reaction for this system with a high Pd loading is illustrated in Figure 3c. If the Pd metal particles grow in size and dominate spatially around the positions of periplasmic hydrogenases and cytochromes, they could interfere with the natural enzymatic electron-transfer pathways and catalytic reactions as a result of their high electronic conductive capacity. An oxidation current of 5.1  $\mu$ A (forward scan) was observed in the presence of formate instead of lactate (Figure 2c). Upon reversal of the potential sweep direction, an oxidation current of 3.2  $\mu$ A was observed. Considering the observed high currents and the profile of the voltammogram, the abiotic electrochemical oxidation of formate on the surfaces of the Pd<sup>0</sup> electrocatalysts is probably due to the intrinsic catalytic activity of the membrane-bound particles; any current from metabolism may then be masked by the abiotic electrochemical oxidation at the nanoparticles themselves.

During potentiostatic investigations, we observed a stable lactate-oxidation current (Figure 2d). The changes in current on the addition of electron donors are shown in Table 1 for a variety of prepared electrodes. In control experiments in which 1) Pd nanoparticles were formed by electrodeposition on the GC surface and 2) an electrode modified with Pd and dead cells was used, no change in the oxidation current was detected upon the addition of lactate. The GC electrode modified with *D. desulfuricans* with a low Pd loading yielded a stable current of 25 nA. This value is a factor of four greater than that observed for the control GC electrode modified with palladium-free *D. desulfuricans*. This result indicates that the Pd nanoparticles bound to the cell membranes may

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

Electrode type	Substrate	Current increase [nA] on substrate addition <sup>[a]</sup>
pristine GC	lactate	n.d.
GC modified with Pd only	lactate	n.d.
<i>D. desulfuricans</i> -GC <sup>[b]</sup>	lactate	n.d.
<i>D. desulfuricans</i> -GC <sup>[c]</sup>	lactate	6.1 $\pm$ 5.3
<i>D. desulfuricans</i> -Pd <sup>0</sup> -GC (low Pd loading) <sup>[c]</sup>	lactate	25 $\pm$ 17
<i>D. desulfuricans</i> -Pd <sup>0</sup> -GC (low Pd loading) <sup>[b]</sup>	lactate	n.d.
<i>D. desulfuricans</i> -Pd <sup>0</sup> -GC (high Pd loading) <sup>[c]</sup>	lactate	n.d.
GC modified with Pd only	formate	191 $\pm$ 87
<i>D. desulfuricans</i> -Pd <sup>0</sup> -GC (high Pd loading) <sup>[c]</sup>	formate	232 $\pm$ 65

[a] The current increase is given with the standard deviation ( $n=6$ ). [b] The electrode was prepared for a control experiment by exposure to air. [c] The electrode was prepared under a nitrogen atmosphere. n.d. = none detected.

facilitate electron transfer. For the GC electrode with *D. desulfuricans* and a high Pd loading, no lactate-oxidation currents were detected, which indicates that the Pd<sup>0</sup> interferes with the microbial enzyme reactions in the cell membrane at this loading. However, the bioparticles can act as highly active catalysts for electrode reactions in the presence of formate and hydrogen, and other redox pairs, and this activity could have potential environmental applications in bioremediation processes.<sup>[19]</sup>

Herein we have presented evidence for a DET mechanism used by *D. desulfuricans* in which periplasmic cytochromes and hydrogenases play an important role. Pd nanoparticles bound to the microbes may participate in the electron-transfer process. The present study is of importance not only for our fundamental understanding of electron transfer in microbial physiology and ecology, but also for the improvement of our understanding of and the performance of bioelectrochemical techniques. For example, precious metals are used extensively as important catalysts and are therefore present in many wastewaters produced by industry.<sup>[19]</sup> Bionanoparticles can oxidize metabolites in situ, for example, H<sub>2</sub>, formate, and ethanol in the anode chambers, while also acting as cathodic catalysts for the oxygen-reduction reaction.<sup>[23]</sup> Furthermore, this study indicates the feasibility of using bioelectrochemical systems for metal immobilization, recovery, or detoxification.

## Experimental Section

**Cultivation:** The *D. desulfuricans* strain was obtained from the German Collection of Microorganisms and Cell Cultures. The cells were grown in sealed bottles for 3 days at 30°C in the medium described previously<sup>[9,10]</sup> and collected by centrifugation at 3500 rpm for 15 minutes. The pellets were washed twice with 20 mM morpholinepropanesulfonic acid (MOPS)-NaOH buffer (50 mL, pH 6.9) to remove any residual sulfide prior to the preparation of the Pd<sup>0</sup> bionanoparticles. All solutions were purged with nitrogen before

use, and anaerobic conditions were maintained throughout the study unless otherwise stated.

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

**Electrode preparation:** After the reduction, the cells were harvested by centrifugation at 3500 rpm for 15 minutes, washed with MOPS buffer and water (> 18.2 M cm<sup>-1</sup>), transferred anaerobically to the surface of a polished glassy-carbon electrode (3 mm diameter), and dried at room temperature under a nitrogen atmosphere. Control experiments were performed with bacteria that had been exposed to air or heat to ensure that they were metabolically inactive, to show that the electric current was produced by the palladium–bacterium species.

**Electrochemical measurements:** Electrochemical characterization was carried out by cyclic voltammetry and potentiostatic measurements by using an Autolab potentiostat/galvanostat with a three-electrode chamber containing a platinum-wire counter electrode and an Ag/AgCl reference electrode. All potentials given herein refer to this reference electrode. A buffer (0.05 M; no culture medium) was used as the electrolyte.

**Electron microscopy and EDX:** Cells suspended in the MOPS buffer were fixed for 1 hour in 2.5 % (w/v) glutaraldehyde and then dehydrated by using an ethanol-in-water series (25, 50, 75, and 100 %) in steps of 15 minutes duration. The suspension was dried in air for 12 hours and then placed on a carbon-coated copper grid for analysis by electron microscopy. A Hitachi HD2300 A scanning transmission electron microscope with an EDX detector was used to characterize the cells and the Pd<sup>0</sup> particles with an acceleration voltage of 200 kV.

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