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Direct Electron Transfer of Cellobiose Dehydrogenase on Positively Charged Polyethyleneimine Gold Nanoparticles

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Abstract: Efficient conjugation between biomolecules and electrode materials is one of the main challenges in the field of biosensors. Cellobiose dehydrogenase (CDH) is a monomeric enzyme, which carries two separate domains; one catalytic dehydrogenase domain (DH_{CDH}) carrying strongly bound flavin adenine dinucleotide (FAD) in the active site and a cytochrome domain (CYT_{CDH}) carrying a *b*-type heme connected by a flexible linker region. Here we report on the development of a lactose biosensor, based on direct electron transfer (DET) from CDH from *Phanerochaete sordida* (PsCDH) electrostatically attached onto polyethyleneimine stabilized gold nanoparticles (PEI@AuNPs) used to cover a conventional polycrystalline solid gold disk electrode. PEI@AuNPs were synthesized in aqueous solution using PEI as reducing agent for Au(III) and as stabilizer for the nanoparticles. The heterogeneous electron transfer (ET) rate (k_s) for the redox reaction of immobilized PsCDH at the modified electrodes was calculated based on the Laviron theory and was found to be $39.6 \pm 2.5 \text{ s}^{-1}$. The proposed lactose biosensor exhibits good long term stability as well as high and reproducible sensitivity to lactose with a response time less than 5 s and a linear range from 1 to 100 μM .

Introduction

In the past decade, the establishment of efficient ET communication between redox enzymes and various electrode materials has been one of the major challenges in the field of bioelectrochemistry both for understanding the structures and mechanism of different enzymatic reactions and for developing bioelectronic devices such as biosensors and biofuel cells^[1]. However, because of too large a distance between the active site and the enzyme surface or unfavorable orientation of the biomolecules at the surface of electrodes, the enzyme

transfer (ET) rate, k_s , although their ET rate is fast in biological systems^[2].

Recently, redox enzymes such as flavocytochromes, quinoxinoproteins, and multicopper oxidases have received much attention in the field of bioelectronic devices, because these multidomain enzymes have both a catalytic domain and a separate ET domain containing another cofactor than the catalytic domain^[3]. The ET domain can act as a built-in mediator^[4] connecting the active site of the catalytic domain with the surface of the electrodes via the ET domain. Cellobiose dehydrogenase (EC 1.1.99.18, CDH) is a monomeric enzymes, which consists of a flavin adenine dinucleotide (FAD) containing dehydrogenase domain (DH_{CDH}) and a *b*-type heme containing cytochrome domain (CYT_{CDH}) connected by a flexible linker region^[5]. Depending on the origin, CDHs show varying substrate specificities, but in general, they all oxidize β -1,4-linked di- and oligosaccharides such as cellobiose, cellodextrins and lactose. In the catalytic cycle, two electrons are donated from the substrate to the oxidized FAD cofactor of DH_{CDH} and will convert FAD into its fully reduced state. In the absence of any electron acceptor, the electrons can be sequentially transferred from the FADH₂ to the *b*-type heme cofactor in the smaller CYT_{CDH} by an intramolecular electron transfer (IET) reaction, which can act as a mediator between DH_{CDH} and an electrode^[6].

To enhance the efficiency of direct electron transfer (DET) of redox enzymes, nanostructured materials such as various kinds of metal nanoparticles^[7], single and multiwall carbon nanotubes^[8] and mesoporous materials^[9] were included between the redox centers of the enzyme and the electrode's surface leading to the advantage of having a large surface area for protein immobilization. The conjugation of enzymes onto surfaces modified with nanomaterials having the opposite charge of the enzyme have been shown to be helpful for the fabrication of biosensors^[10]. Some of the approaches for providing cationic charges on metals and metal oxide nanoparticles, which include silica, iron oxide, silver and gold nanoparticles, typically involve surface coating with cationic polymers (e.g., polyethyleneimine, polylysine, and polyallylamine hydrochloride) through either electrostatic or covalent conjugation^[11]. PEI is a synthetic polycation consisting of a high density of cationic groups such as secondary and tertiary amines, which make PEI completely miscible with water at room temperature. PEI is often used in biosensor constructions due to its positive charges at neutral pH and adhesive properties^[12]. Recently, Schulz *et al.* have shown that the catalytic currents from lactose oxidation by spectroscopic graphite electrodes modified with CDH from *Myriococcum thermophilum* (MtCDH) could be highly increased through modification of the electrodes with branched PEI^[13]. It is suggested that the increase in the catalytic currents could be accomplished from either facilitated IET due to adjustment of the distance between the CYT_{CDH} and DH_{CDH} domains through the positive charges of the polycation or

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molecules may exhibit a rather low heterogeneous electron

by increasing the surface loading as a result of premodification of the electrode surface with PEI. Diazonium activation of single walled carbon nanotubes yielding a highly positively charged electrode surface was shown to largely increase the catalytic current density for *Phanerochaete sordida* CDH (PsCDH) modified electrodes.^[14] Matsumura *et al.* have also reported DET reaction of *Phanerochaete chrysosporium* CDH (PcCDH) covalently immobilized on AuNPs modified electrodes. They have shown that the catalytic current density of PcCDH/AuNPs electrodes was notably enhanced compared with those electrodes without modification with NPs^[7d]. In general our observations using various AuNP modified electrodes show a very beneficial improvement of the DET properties of various CDHs and electrodes.^[6b, 7d, 15]

In this study, we report on combining our previous observations to improve the DET reaction of PsCDH electrostatically attached to gold electrodes premodified with positively charged polyethyleneimine coated AuNPs. The AuNPs were synthesized in aqueous solution using PEI as reducing agent for the metal precursor and as stabilizer for the final NPs. PEI has recently been shown to be beneficial for the construction of CDH based biosensors by enhancing the surface load of CDH and possibly the rate of the IET between DH_{CDH} and CYT_{CDH}^[13]. After immobilization of PsCDH, the biosensor exhibited clear, well defined redox waves of the heme *b* of CYT_{CDH}. The long term stability and catalytic current density of the proposed biosensor using lactose as substrate was drastically increased compared with unmodified solid Au electrodes.

Results and Discussion

Characterization of gold nanoparticles. An appealing feature of AuNPs is the possibility to change their properties by varying the size of the core as well as the surface modification with suitable functional molecules. Although the surface of AuNPs can be easily functionalized with a polymer shell by layer-by-layer assembly of certain polyelectrolytes^[16], in this work we utilize a one-step synthesis method developed recently by Sun *et al.*, who used PEI as both the reducing agent and stabilizer to prepare PEI-capped AuNPs^[10a, 11a, 17]. After directly mixing the metal precursor solution with PEI at room temperature, the color of the reaction mixture turns to dark red after 24 h, indicating the formation of AuNPs. As shown in Fig. 1a, the UV-vis absorption maximum of the resulting AuNPs appeared at 527 nm due to the surface plasmon resonance (SPR) of the AuNPs, which confirmed the formation of AuNPs-PEI^[17-18]. This corresponds to a 5 nm diameter using the Haiss' numerical method based on the quotient of the absorption at the surface plasmon resonance peak and the absorption at 450 nm^[19]. This is lower than the value obtained from the TEM measurements, see below. The underestimation using the Haiss' method might originate from the PEI modification of the nanoparticles partially quenching the surface resonance peak. The diameter of the prepared, spherical AuNPs-PEI was 9±2 nm measured using transmission electron microscopy (TEM) images, Fig. 1c. The hydrodynamic diameter determined by dynamic light scattering (DLS), 11±1 nm,

is larger (data not shown). Due to the fact that TEM images display only the metal core, it can be assumed that the AuNPs are surrounded by a polyethyleneimine shell of around 2–3 nm. Cyclic voltammograms of Au and PEI@AuNPs/Au electrodes were recorded in 0.5 M H₂SO₄ solution at a scan rate of 100 mV s⁻¹. The CV shows (Fig. 1b) the characteristic current features for formation of Au oxide at 1.3 V and rereduction of the Au oxide to Au at 0.83 V^[20]. The typical responses corresponding to the so-called surface redox reaction of gold were observed. It can be seen that the effective surface area of the PEI@AuNPs/Au electrodes is about 30 times higher than that of unmodified gold electrodes, which is due to the modification of AuNPs onto the Au electrodes, leading to the increase in surface area of the modified electrodes. The binding of the PEI@AuNPs to the gold surface might be due to interactions of the hydrophobic carbon chains of the PEI with the hydrophobic gold surface^[21] or due to a direct hydrophobic gold-gold interaction of partially uncoated PEI@AuNPs with the gold surface.

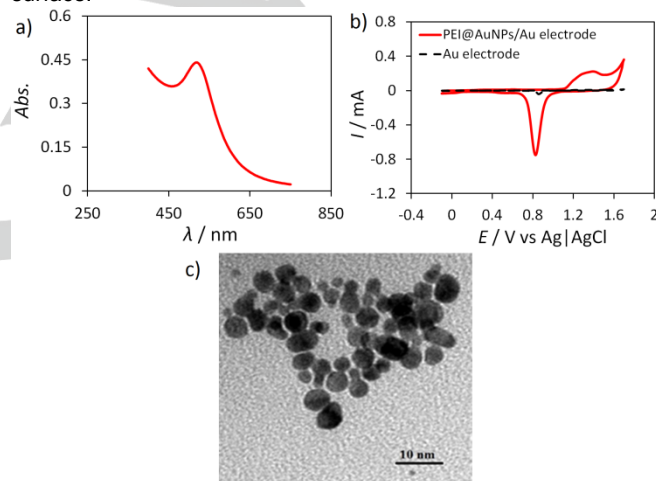


Figure 1. a) UV-VIS spectrum of PEI@AuNPs. b) CVs of unmodified Au electrode (dashed line) and PEI@AuNPs/Au electrode (red line) in 0.5 M H₂SO₄ solution. Scan rate: 100 mV s⁻¹. c) TEM of PEI@AuNPs.

Electrochemistry of PsCDH on PEI@AuNPs modified gold electrodes.

To study the DET of PsCDH electrostatically bound to PEI@AuNPs modified gold electrodes, we initially investigated CVs obtained for the enzyme in the absence and in the presence of the PEI-capped AuNPs. As shown in Fig. 2a, in the presence of PEI@AuNPs, the PsCDH modified electrode exhibits a pair of clear well-defined and reversible redox waves in acetate buffer (pH 4.5), corresponding to the DET communication between the CYT_{CDH} domain of immobilized PsCDH and the AuNPs^[7d, 22]. No significant waves of the heme of the CYT_{CDH} were observed in the absence of the PEI@AuNPs (Fig. 2a). The presence of clear redox waves for PsCDH when using the PEI@AuNPs could be explained by an increased number/surface concentration of well oriented PsCDH molecules immobilized on the electrode surface being capable of efficient DET, as previously suggested^[23]. Electrostatic interactions between the positively charged PEI@AuNPs and negatively

charged *PsCDH* (pI 4.1) at pH 4.5 may play a major role. On the other hand, the surface modification of the solid Au electrode with the NPs created a really porous structure. The effective surface area of the AuNPs modified electrode was enhanced 30 times in comparison to the geometric surface of the bare electrode estimated from CVs of Au and PEI@AuNPs/Au electrodes in 0.5 M H_2SO_4 solution (Fig. 1c). The effective immobilization of enzyme and the porosity of the AuNPs coated surface are combined to assist the progress of DET communication between the heme *b* group of the enzyme and surface of the electrode. The formal potential (E°) of the heme *b* group of CYT_{CDH} , evaluated as the midpoint of the oxidation and reduction peak potentials, was approximately -25 mV vs. $\text{Ag}|\text{AgCl}$ (sat. KCl), which is consistent with previously reported values for *PcCDH* in solution^[24]. The peak-to-peak potential separation (ΔE_p) and the ratio of the anodic to cathodic peak currents were approximately 55 mV and 1, respectively.

Figure 2b presents typical CVs for the *PsCDH* modified PEI@AuNPs gold electrodes in an acetate buffer solution (5 mM, pH 4.5) at different scan rates. The anodic and cathodic peak currents were linearly proportional versus the scan rate, ν , in the range of 1-300 mV s^{-1} (Fig. 2c), indicating that this was a surface controlled electrochemical behavior.

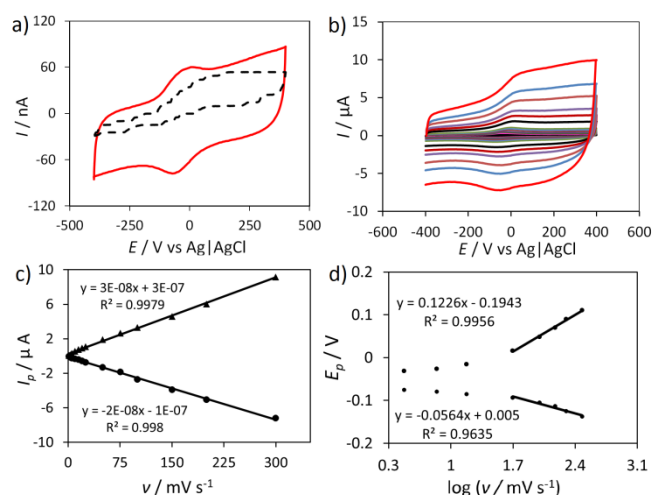


Figure 2. (a) CVs of the *PsCDH*/PEI@AuNPs/Au (red line) and the *PsCDH*/Au (dotted line) in a N_2 -saturated acetate buffer solution (5 mM, pH = 4.5) at a scan rate of 1 mV s^{-1} . (b) CVs of the *PsCDH*/PEI@AuNPs/Au in a N_2 -saturated 5 mM acetate buffer solution at pH 4.5 at various scan rates from 1 to 300 mV s^{-1} . (c) Plot of the peak current (I_p) versus scan rate (ν). (d) Variations of the ($E_p - E^\circ$) vs. the logarithm of the scan rate ($\log \nu$) for a *PsCDH* modified electrode.

Further analysis of the CVs yields the apparent heterogeneous electron transfer rate constant (k_s) for the redox reaction of the immobilized CDH with the modified PEI@AuNPs electrode using Laviron's theory^[25]. The graph of the peak potentials as a function of the logarithm of scan rate (Fig. 2d) shows a typical trumpet shape, in which at high scan rates the peak potentials separate from the E° value with a slope of 120 mV/log unit due

to limitations of the charge transfer kinetics. The so determined α value was 0.55. The k_s could also be calculated based on the Laviron equation (1), which was 39.6 (± 2.5) s^{-1} (ν changed from 50 to 300 mV s^{-1}).

$$\log k_s = \left[(\alpha \log [(1-\alpha) + (1-\alpha) \log [(RT/nF\nu)]] - \alpha(1-\alpha)nF\Delta E_p) / 2.3RT \right] \quad (1)$$

The k_s registered for modified PEI@AuNPs electrodes is higher compared with the intramolecular ET rate between the DH_{CDH} and the CYT_{CDH} domains (30 s^{-1}), which has been suggested to be the rate-limiting step in the overall *PsCDH* reaction^[26]. Thus, the maximum electrocatalytic current would be limited by the intrinsic enzymatic dehydrogenase activity on the electrodes.

In previous approaches various gold and carbon based electrode materials were used also combined with nanoparticles, nanotubes, polycations and self-assembled monolayers reporting on similar CDH modified electrodes/biosensors. The most recent review on such various modifications is given in Ludwig et al 2013^[6b]. The advantage of the here chosen approach is the combination of the nanoparticles, which enhance the active electrode surface area, with polycations enhancing the enzyme load as well as the rate of direct and internal electron transfer. The easiness of nanoparticle and CDH immobilization by hydrophobic and electrostatic interactions compared to more elaborate binding/crosslinking schemes is another benefit.

The obtained rate of 39.6 (± 2.5) s^{-1} is lower compared to when *P. chrysosporium* CDH was covalently immobilized onto SAM modified gold electrodes as reported in Matsumura et al. 2012^[7d]. This might be explained due to the insulating, non-conducting characteristics of PEI (and that there might be differences between *PsCDH* and *PcCDH* in their respective electron transfer characteristics).

Electrocatalytic behavior of *PsCDH* modified PEI@AuNPs gold electrodes. As mentioned above, all CDHs are monomeric enzymes, which carry a flavin adenine dinucleotide (FAD) containing dehydrogenase domain DH_{CDH} and a *b*-type heme containing CYT_{CDH} . In the catalytic cycle, the DH_{CDH} catalyzes the dehydrogenation of cellobiose, cello-oligosaccharides, and lactose to their corresponding δ -lactones followed by intermolecular electron transfer (IET) from DH_{CDH} to CYT_{CDH} . In this work, lactose was chosen as substrate because lactose does not exhibit substrate inhibition for *PsCDH* in contrast to the natural substrate, cellobiose^[27]. Figure 3 presents the CVs of the *PsCDH* modified PEI@AuNPs gold electrodes in acetate buffer solution containing different concentrations of lactose from 0.0 to 5 mM. In the absence of substrate, the redox peaks of the heme *b* group can be observed at around -20 mV. After addition of lactose, the catalytic current appears at the beginning of the anodic peak of heme *b* of the CYT_{CDH} , indicating that the CYT_{CDH} is responsible for electro-chemical communication between *PsCDH* and the electrode so that the oxidation of lactose can be followed and registered. The electrocatalytic current density is around 10 $\mu\text{A cm}^{-2}$ for 5 mM lactose and a scan rate of 1 mV s^{-1} in acetate buffer.

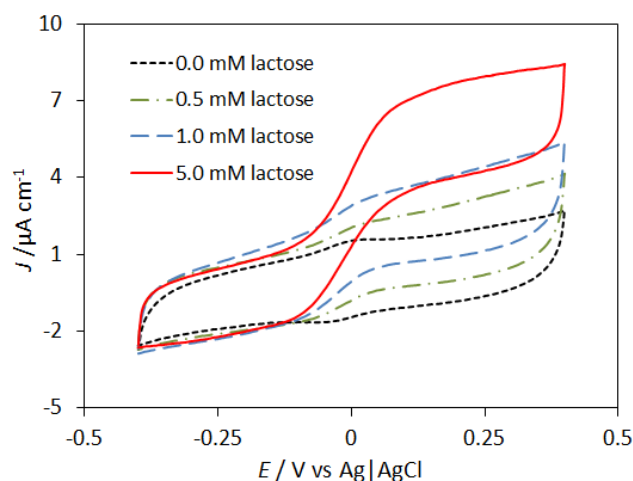


Figure 3. Electrocatalytic currents of *PsCDH* electrostatically immobilized on modified PEI@AuNPs electrode in the presence of 0.0, 0.5, 1.0 and 5.0 mM lactose. The experiments were performed at a scan rate of 1 mV s^{-1} in a N_2 -saturated 5 mM acetate buffer solution at pH 4.5.

Flow injection analysis. Flow injection measurements were performed with *PsCDH* modified PEI@AuNPs gold electrodes mounted in a flow through electrochemical cell^[28] coupled to a single line flow injection analysis (FIA) system. The applied potential for all *PsCDH* modified electrodes was 250 mV vs. Ag|AgCl (0.1 M KCl) reference electrode, i.e., positive enough to guarantee that the ET from *PsCDH* bound to the PEI@AuNPs modified gold electrodes is not the kinetically limiting step. The peak response is fast with around 3 s, mainly because of the good communication between the *PsCDH* and the surface of the electrodes.

A calibration curve for the *PsCDH* modified PEI@AuNP gold electrodes done with different concentrations of lactose in acetate buffer (5 mM, pH 4.5) is shown in Fig. 4. The calibration curve was linear in the concentration range of 1–100 μM , with a correlation coefficient (R^2) of 0.99. The detection limit was found to be 0.330 μM ($S/N = 3$). The relative standard deviation for repeated measurements ($n = 6$) for 5 mM lactose was 4.46%. As shown in Table 1, the analytical characteristics of some reported lactose biosensors are summarized and compared with those obtained in this work using *PsCDH*/PEI@AuNPs solid gold electrode. The results confirmed that the proposed biosensor has good performing properties such as low detection limit, fast response and acceptable linear range due to the enhancement of the electroactive surface area as well as excellent communication between the *PsCDH* molecules and the positively charged AuNPs.

Table 1. Analytical parameters reported for some lactose biosensors

Lactose biosensor	Response Time (s)	DL ^[a] (μM)	Sensitivity ($\mu\text{A mM}^{-1}$)	E_{app} (V)
(PEI/ β -Gal) ^[b] / [(PEI/PVS ^[c])/PB ^[d]]/ITO ^[e] [12e]	-	1130	0.04	0.04

β -Gal-GaOx/PPy ^[f] /Pt ^[29]	8–10	2	76	0.04
β -Gal-GOD ^[g] - HRP ^[h] /TTF ^[i] /MPA ^[j] /AuE ^[30]	300	3.8	11	0.00
<i>PsCDH</i> /MWCNTs/SPCEs ^[k] [31]	65	0.25	-	0.1
<i>PsCDH</i> /PEI@AuNPs/AuE	3	0.33	3.93	0.25

[a] detection limit. [b] β -galactosidase. [c] poly (vinyl sulfonate). [d] Prussian Blue. [e] indium tin oxide. [f] polypyrrole. [g] glucose oxidase. [h] horseradish peroxidase. [i] tetrathiafulvalene. [j] 3-mercaptopropionic acid. [k] screen printed carbon electrodes.

The apparent Michaelis-Menten constant (K_M^{app}) was estimated by fitting the calibration curves illustrated in Fig. 4 to the Michaelis-Menten equation. The observed K_M^{app} value of *PsCDH* for lactose was determined to be 0.2 mM, which was smaller than that obtained when immobilized onto aryl diazonium modified single wall carbon nanotubes deposited on a glassy carbon electrode (0.7 mM) indicating less mass-transfer resistance on the *PsCDH* modified PEI@AuNP gold electrodes^[14] [32].

Figure 5 shows the dependence of the catalytic currents gained from the oxidation of lactose by the *PsCDH* modified PEI@AuNP gold electrodes versus the solution pH, which was determined amperometrically using the FIA system. The pH optimum was found to be at pH 4.0, which was similar to previous reported results^[32].

Stability Measurements. The stability of the *PsCDH* modified PEI@AuNP gold electrodes was measured using the amperometric flow injection analysis system by measuring the decrease in the electrocatalytic currents during successive injections of lactose every 5 min. The catalytic currents decreased only by 5.3% during approximately 300 continuous injections for 24 h. Attractive electrostatic interactions between the negative residues of *PsCDH* and the positive shell around the PEI@AuNPs seem to prevent the release of the enzyme from the surface of PEI@AuNPs electrodes and support the long term stability of the biosensors.

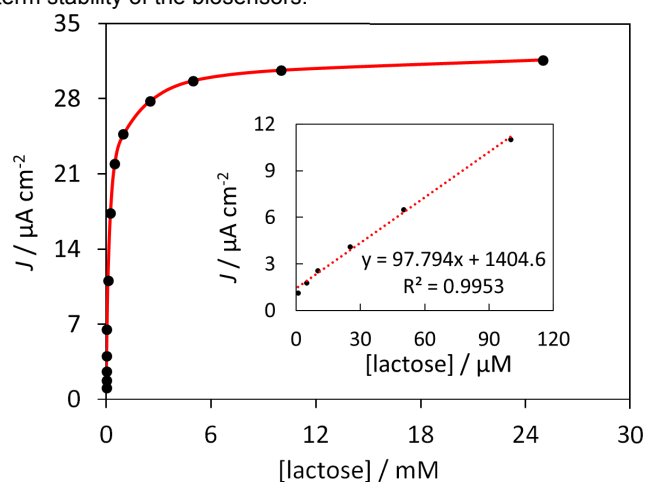


Figure 4. Calibration curve for different concentrations of lactose in buffer acetate (5 mM, pH 4.5). In the inset: the response in the low-micromolar range. Applied potential: +250 mV vs. Ag|AgCl (0.1 M KCl).

Conclusions

We have developed an approach for lactose biosensing, based on DET of electrostatically attached *PsCDH* onto gold electrodes premodified with PEI capped gold nanoparticles synthesized in aqueous solution using PEI as reducing agent for the Au(III) and as stabilizer for the nanoparticles. Basic characterization of the AuNPs by TEM and DLS reveal a gold core particle diameter of 9 ± 2 nm capped by a polyethyleneimine shell of around 2–3 nm. Clear, well defined redox waves due to the electrochemical communication with the CYT_{CDH} domain of *PsCDH* was observed in CV when *PsCDH* was electrostatically attached on the surface of the PEI@AuNP modified gold electrodes, whereas the corresponding *PsCDH* modified polycrystalline gold electrodes lacking the PEI@AuNP did not show any redox waves. The heterogeneous ET rate (k_s) could also be estimated based on the Laviron approach and was found to be $39.6 (\pm 2.5) \text{ s}^{-1}$.

The proposed lactose biosensor presented a number of attractive features such as a wide linear range for the detection of lactose between 1 – 100 μM , a fast current response and an excellent long term stability with only a 5% signal loss in 24 h. These features can be attributed to a higher number of well oriented enzyme molecules immobilised on the PEI@AuNP modified electrode surface due to electrostatic interactions between the negatively charged *PsCDH* molecules and the positively charged, PEI capped gold nanoparticles.

Experimental Section

Reagents and Equipment. The metal precursor, HAuCl_4 , branched polyethylenimine (PEI, 50% w/v in H_2O , $M_n \sim 60\,000$, $M_w \sim 750\,000$), β -lactose, 4-morpholineethanesulfonic acid (MES) and tris(hydroxymethyl)aminomethane (TRIS) were obtained from Sigma Aldrich Chemicals (Steinheim, Germany). Acetic acid (100%) and sodium

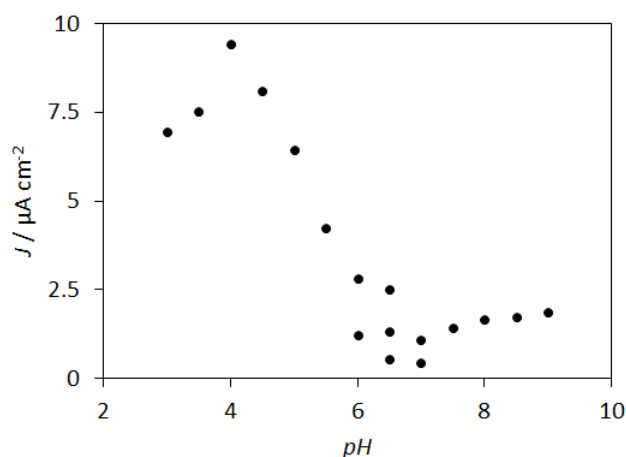


Figure 5. Dependence of catalytic currents versus pH-profile. The electrodes were mounted in an electrochemical flow through cell connected to a FIA system with 50 mM NaAc, MES, or TRIS of varying pH as a running buffer. The electrocatalytic current responses to 5 mM lactose were recorded at an applied potential of 250 mV vs. Ag|AgCl (0.1 M KCl).

hydroxide solution (50%) were purchased from Merck (Darmstadt, Germany). All chemicals were of analytical reagent grade and were used without further purification. Milli-Q water (Millipore 18.2 M Ω cm, Millipore, Bedford, MA, USA) was used in all experiments.

Cyclic voltammetry studies were performed using an electrochemical analyzer model BAS 100 (Bioanalytical Systems, West Lafayette, IN, USA) with a conventional three electrode setup, in which a *PsCDH*/PEI@AuNPs/Au electrode, an Ag|AgCl (saturated KCl) and a platinum foil served as the working, reference and auxiliary electrodes, respectively. The experiments were performed at a scan rate of 1 mV s^{-1} in a N_2 -saturated 5 mM acetate buffer solution at pH 4.5. For amperometric studies a wall jet cell^[28] with three electrodes connected to a potentiostat (Zäta Elektronik, Höör, Sweden) was used containing a modified Au electrode as working electrode, Ag|AgCl (0.1 M KCl) as reference electrode and a Pt wire as auxiliary electrode. 5 mM acetate buffer (pH 4.50) was used as carrier buffer with a constant flow rate of 0.5 mL min^{-1} by a peristaltic pump (Minipuls 2, Gilson, Villier-le-Bel, France). An electrically controlled six-port valve (LabPRO, Rheodyne, Cotati, CA, USA) with a 50 μL injection loop was used for the injection of analytes into the carrier flow.

The hydrodynamic radius of the AuNPs was measured using a Nano Zetasizer equipped with a He–Ne laser ($\lambda = 633 \text{ nm}$) and a digital autocorrelator at room temperature.

Solutions and enzymes. The protein concentration of *PsCDH* was calculated photometrically by using an extinction coefficient of $\epsilon_{280} = 141750 \text{ M}^{-1} \text{ cm}^{-1}$ determined from the amino acid sequence (ABS45567.2). A stock solution of lactose (500 mM) was prepared using Milli-Q water and stored at 4°C when not in use. The lactose stock solution was allowed to mutarotate for at least 24 h before use. The running buffers were 5 mM sodium acetate (pH 3.0–6.5), 5 mM MES (pH 6.0–7.0) and 5 mM TRIS (pH 6.5–9.0).

Synthesis of gold nanoparticles. 0.01 g of branched PEI was dissolved completely in 100 mL of distilled water for an hour. Then, HAuCl_4 was dissolved in the aqueous solution of PEI to a final concentration of 2 mM under magnetic stirring for another hour. The mixture was stirred vigorously at room temperature for 24 h for complete reduction. In this case, PEI played a role of a mild reductant so that a slow colour change from yellow to red was observed, indicating the formation of AuNPs. Then, the reacted mixture was dialyzed using a membrane with a molecular weight cut off of 12 kDa with repeated water exchanges for 1 day to eliminate remaining unreacted chemicals.

Preparation of *PsCDH* immobilized on PEI@AuNPs modified gold electrodes. The surface of polycrystalline gold disk electrodes (BAS, West Lafayette, IN, USA, $d = 0.016 \text{ cm}$) was mechanically polished on a Microcloth (Buehler, Lake Bluff, IL, USA) in aqueous alumina FF slurry ($d = 0.1 \mu\text{m}$, Struers, Copenhagen, Denmark), then thoroughly rinsed with Milli-Q water, followed by ultrasonication for 5 min in Milli-Q water. The electrodes were electrochemically cleaned by cycling in $0.5 \text{ M H}_2\text{SO}_4$, with a scan rate of 100 mV s^{-1} between -300 and $-1700 \text{ mV vs Ag|AgCl}$ (saturated KCl) for 20 scans and finally rinsed with Milli-Q water.^[33] To obtain AuNPs modified solid gold electrodes, a volume of $5 \mu\text{L}$ of the concentrated AuNPs solution was cast on the surface of the clean gold

electrode and dried at room temperature. Modification of the electrode with *PsCDH* was made by trapping a 3 μ l droplet of the enzyme solution (10 mg ml^{-1}) between the gold electrode surface and a permselective dialysis membrane (MWCO < 12 000–14 000 Da, Spectrum Laboratories, Broadwick, CA, USA). The prepared electrodes were immersed into 50 mM acetate buffer (pH 4.5) for 1 h, and then the membrane was removed, followed by rinsing with buffer.

Acknowledgements

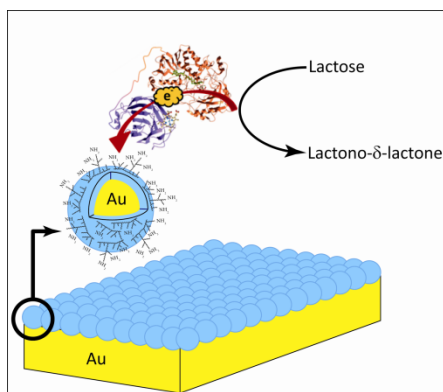
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Keywords: Lactose biosensor; Polyethyleneimine gold nanoparticles; Direct electron transfer; Cellobiose dehydrogenase; Lactose.

ARTICLE

Here we report on the development of a lactose biosensor, based on direct electron transfer (DET) from CDH from *Phanerochaete sordida* (PsCDH) electrostatically attached onto polyethyleneimine gold nanoparticles (PEI@AuNPs) used to cover a conventional solid gold disk electrode.



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