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Bioelectrocatalytic reduction of oxygen at gold nanoparticles modified with laccase

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ABSTRACT

To characterise bioelectrocatalytic oxygen reduction at gold nanoparticles (AuNPs) modified with *Trametes hirsuta* laccase (*Th*Lc) combined electrochemical and quartz crystal microbalance measurements have been used. The electrodes with different degrees of AuNP-monolayer coverage, θ, have been studied. In every case of θ close to theoretically possible 44 *Th*Lc molecules adsorbed at 22 nm diameter AuNP. The bioelectrocatalytic current was recalculated down to the current at a single AuNP. Unexpectedly, the current at a single AuNP was higher when θ was higher. The maximum current reached at a single AuNP was $31 \cdot 10^{-18}$ A which corresponds to the enzyme turnover (k_{cat}) 13 s^{-1}. This rate is lower than the homogeneous *Th*Lc turnover (190 s^{-1}) suggesting partial denaturation of *Th*Lc upon adsorption or that some *Th*Lc are not in DET contact with the electrode surface.

1. Introduction

Since the discovery of direct electron transfer (DET) between the redox enzymes and electrodes [1] it has been intensively studied [2–4] and applied to design biosensors [5,6] and biofuel cells (BFCs) [7–12]. It has been found that the majority of enzymes show slow DET which limits their application for the development of the above-mentioned biodevices or restricts the efficiency and stability of the device. A number of studies have proved that DET of redox enzymes could be improved by exploiting gold nanoparticles (AuNPs) [13–17]. The observed enhancement of DET can be simplistically explained by the fact that nanoparticle (NP) comes into close contact with the active centre of an enzyme and functions as an intermediate electron hopping site in the heterogeneous ET pathway [18]. We have recently demonstrated [17] that AuNPs enable DET based bioelectrocatalytic oxygen reduction (Fig. 1) by the laccase from basidiomycete *Trametes hirsuta*.

The laccase (Lc) is a blue multicopper oxidase with the active centre comprised of four copper ions classified as T1, T2 and T3 [19]. During DET based oxygen bioelectroreduction the electrons are transferred from the AuNP modified electrode surface to the T1 copper site and further onto the T2/T3 copper cluster where the oxygen is reduced to water (Fig. 1). It should be noted that the mechanism of DET for this enzyme adsorbed at carbon based electrodes is relatively well understood [2], while it is still debatable in case of metal electrodes, *e.g.*, gold [20,21]. To understand and implement bioelectrochemical reduction of oxygen at Lc modified electrodes is of high practical interest for designing BFCs [9,22]. BFC is a device which converts the energy conserved in organic and inorganic materials into electrical energy with the help of enzymes or living cells [23,24]. In this context one of the obvious aims is to develop high current density enzymatic FCs based on DET reactions. This aim can be reached by loading a high amount of redox enzymes into electrically conducting three-dimensional (3D) nanomaterials [16,25,26]. Examples of BFC electrodes based on DET of Lcs [27], bilirubin oxidases [16], peroxidases [28], fructose dehydrogenases [29], *etc.*, have been demonstrated in numerous studies. Colloidal AuNPs are often used in BFC design [30,31], however, other 3D gold nanostructures [28,32] have also been investigated. Although BFC cathodes based on 3D AuNP assembly function relatively well [11,16,30], DET between the redox enzyme and the AuNP in these structures is difficult to characterise. The knowledge is important, *e.g.*, for miniaturising BFCs. Thus, DET studies of the laccase from *T. hirsuta* (*Th*Lc) at AuNPs have been addressed in this work, especially, being aware that stable and high current density providing 3D biocathodes can be assembled by using Lc and AuNP building blocks. The purpose of the present investigation was to answer the questions: (i) How many *Th*Lc molecules adsorb at a single AuNP? (ii) What is an approximate current magnitude of oxygen bioelectroreduction at a single AuNP modified with *Th*Lc? (iii) What is the rate of the limiting reaction which defines oxygen bioelectroreduction at the AuNP-*Th*Lc structure? To answer these questions we have conducted combined quartz crystal microbalance with dissipation (QCM-D) and electrochemical study of AuNPs modified by *Th*Lc.

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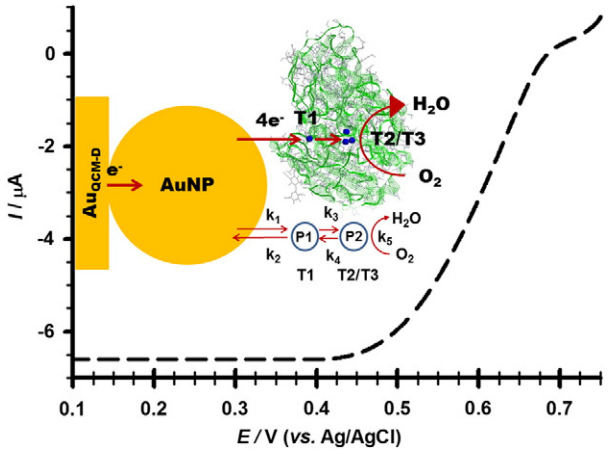

Fig. 1. The schematic representation of DET between a Lc molecule (green ribbons) and the AuNP modified planar gold electrode. The T1 copper site and the T2/T3 copper cluster in Lc are presented as blue dots. Red arrows represent the direction of ET and the reaction of oxygen reduction to water at the applied potential below 650 mV (*vs.* Ag/AgCl/3 M KCl). The scheme represents a sketch summarising the mechanism of bioelectroreduction of oxygen at the electrode as described by Eqs. (4)–(6).

2. Experimental

2.1. Chemicals and materials

$Na_2HPO_4 \cdot 2H_20$, KCl, NaCl, Na_2SO_4, $HAuCl_43H_20$, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) and citric acid monohydrate were purchased from Sigma (St. Louis, MO, USA). Trisodium citrate-2-hydrate and NaF were obtained from Merck (Darmstadt, Germany). All chemicals were of analytical grade. Poly-L-lysine (PLL) with the molecular weight of 70–150 kDa (for calculations the average MW of 110 kDa was taken), 0.01% w/v in water, was received from Sigma-Aldrich (Irvine, Ayrshire, UK). Buffers and all other solutions were prepared using deionized water (18.2 MΩ cm) purified with PURELAB UHQ II system from ELGA Labwater (High Wycombe, UK).

The dispersion of spherical AuNPs with an average NP diameter of 22 ± 4 nm was prepared by reducing $HAuCl_4$ with sodium citrate [33] and dialyzing against water. Their mean diameter and standard deviation was determined by recording NP diameter distribution using atomic force microscopy after drying their dispersion on mica surface pretreated with PLL. The dispersion was stored in glass bottles at +4 °C when not in use. As verified by SDS/PAGE a homogeneous preparation of fungal Lc (EC 1.10.3.2) from basidiomycete *T. hirsuta* with molecular mass of 70 kDa (glycosylation 12%, pI 4.2) was used [34]. The stock solution of *Th*Lc with 14.4 mg/ml enzyme concentration in 0.1 M phosphate buffer, pH 6.5, was stored frozen at −18 °C. For the experiments the stock solution of the enzyme was thawed at +4 °C and diluted to 0.7 mg/ml concentration. The *Th*Lc activity and electrochemical measurements were performed in 50 mM phosphate buffer (pH 4.0) containing 0.1 M Na_2SO_4 (adjusted by citric acid). The appearance of a coloured product during the oxidation of ABTS catalysed by *Th*Lc was exploited to evaluate the activity (turnover number) of *Th*Lc [35]. The homogeneous turnover (k_{cat}) of the enzyme was 190 s^{-1} at room temperature. AT-cut gold-coated quartz crystals (QSX 301) with a fundamental frequency of 4.95 MHz were purchased from Q-Sense (Gothenburg, Sweden). The geometric area of the gold surface was 0.785 cm^2, which was similar to the electrode area determined by cyclic voltammetry experiments (see supplementary materials).

2.2. QCM-D and electrochemical measurements

To quantify AuNPs and *Th*Lc molecules attached to the surface, and to evaluate the bioelectrocatalytic properties of the AuNP-Lc layer on a planar gold electrode, simultaneous electrochemical and QCM-D measurements were performed using the QWEM401 electrochemistry cell (Q-Sense, Gothenburg, Sweden). All measurements were made at +22 ± 0.02 °C. The planar surface of gold covered QCM-D sensor ($Au_{QCM\text{-}D}$) was modified with PLL, AuNPs and the enzyme by the following procedure. PLL was adsorbed on the QCM-D sensor and then *Th*Lc was deposited. The resulting structure was noted as $Au_{QCM\text{-}D}$-PLL-*Th*Lc. Afterwards, the AuNP layer was adsorbed following by adsorption of *Th*Lc ($Au_{QCM\text{-}D}$-PLL-*Th*Lc-AuNPs-*Th*Lc). We assumed that this final *Th*Lc adsorption step resulted into the enzyme adsorption only on AuNPs since adsorption of *Th*Lc directly on PLL covered gold sensor was already blocked by the first *Th*Lc adsorption step. The conditions for each adsorption step as well as the calculation of adsorbed mass are described in the supplementary materials.

After each adsorption step, *i.e.*, for QCM-D electrode structure denoted as $Au_{QCM\text{-}D}$-PLL-*Th*Lc, $Au_{QCM\text{-}D}$-PLL-*Th*Lc-AuNPs, and $Au_{QCM\text{-}D}$-PLL-*Th*Lc-AuNPs-*Th*Lc, the electrode was assessed by linear sweep voltammetry (LSV) at 1 mV/s potential scan rate using compactstat from IVIUM (Eindhoven, Netherlands). Ag/AgCl/3 M KCl inserted into the outlet of the QCM-D flow cell served as a reference and a sputtered ring of platinum on the glass window of the cell as a counter electrode, respectively. The volume above the sensor was about 100 μl. LSVs were recorded under the flow (100 μl/min) of the buffer solution in the QCM-D cell.

3. Results and discussion

To characterise *Th*Lc interaction with AuNPs and assess bioelectrocatalytic properties of the AuNP-*Th*Lc nanocomponent we carried out simultaneous QCM-D and LSV measurements. AuNPs were assembled on a planar gold surface of the QCM-D sensor, $Au_{QCM\text{-}D}$, with subsequent adsorptive modification by *Th*Lc. From our previous investigations we know that such an assembly enables considerable, DET based, bioelectrocatalytic reduction of oxygen (Fig. 1) at applied potential higher than the formal potential of the T1 copper of *Th*Lc [17]. However, as a number of *Th*Lc molecules adsorbing and generating the bioelectrocatalytic current at a single AuNP are unknown it is difficult to understand the limitations and improvement possibilities of bioelectrocatalysis at the AuNP-*Th*Lc assemblies. The mentioned characteristics, at least to some degree, could be assessed by using methods applied to measure surface bound mass (*e.g.*, QCM-D) and the reduction current simultaneously.

The mass was monitored for surface structures obtained by the following adsorption sequence: $Au_{QCM\text{-}D}$-PLL (PLL adsorbed on the QCM-D sensor surface), $Au_{QCM\text{-}D}$-PLL-*Th*Lc (PLL adsorption was followed by *Th*Lc adsorption), $Au_{QCM\text{-}D}$-PLL-*Th*Lc-AuNPs (AuNPs were adsorbed on PLL-*Th*Lc treated QCM-D sensor surface), and $Au_{QCM\text{-}D}$-PLL-*Th*Lc-AuNPs-*Th*Lc (*Th*Lc was adsorbed on surface modified by PLL-*Th*Lc-AuNPs). We should mention that notations of surface structures, *e.g.*, $Au_{QCM\text{-}D}$-PLL-*Th*Lc-AuNPs-*Th*Lc, reflect rather the sequence of the surface modification than the real layer structure, which is obviously more disordered. It was experimentally found that AuNPs did not adsorb on the *Th*Lc modified planar $Au_{QCM\text{-}D}$ electrode if the modification with PLL was omitted. These experiments revealed that AuNP assembly is due to the AuNP interaction with PLL layer. The PLL-AuNP interaction was also negligibly affected by the *Th*Lc layer adsorbed on $Au_{QCM\text{-}D}$-PLL surface structure since there was no noticeable difference in the amount of adsorbed AuNPs on $Au_{QCM\text{-}D}$-PLL and $Au_{QCM\text{-}D}$-PLL-*Th*Lc surface. This indicates that the majority of *Th*Lc molecules adsorb on PLL non-covered planar $Au_{QCM\text{-}D}$ sensor surface. Due to the importance of PLL for the assembly of the AuNP-*Th*Lc layer, PLL adsorption is discussed in supplementary materials concluding that the amount of adsorbed PLL on $Au_{QCM\text{-}D}$ surface was equal to 0.8 ± 0.2 pmol/cm^2.

3.1. Sequential adsorption of Lc, AuNPs and Lc

After the PLL layer was adsorbed on $Au_{QCM\text{-}D}$ sensor and rinsed with water the *Th*Lc solution was pumped through the QCM-D flow cell.