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Direct, Electrocatalytic Oxygen Reduction by Laccase on Anthracene-2-methanethiol Modified Gold

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Abstract

Laccase, a multicopper oxidase, catalyses the four electron reduction of oxygen to water. Upon adsorption to an electrode surface, laccase is known to reduce oxygen at overpotentials lower than the best noble metal electrocatalysts usually employed. While the electrocatalytic activity of laccase is well established on carbon electrodes, laccase does not typically adsorb to better defined noble metal surfaces in an orientation that allows for efficient electrocatalysis. In this work, we utilized anthracene-2-methanethiol (AMT) to modify the surface of Au electrodes and examined the electrocatalytic activity of adsorbed laccase. AMT facilitated the adsorption of laccase, and the onset of electrocatalytic oxygen reduction was observed as high as 1.13 V_{RHE}. We observed linear Tafel behavior with a 144 mV/dec slope, consistent with an outer sphere single electron transfer from the electrode to a Cu site in the enzyme as the rate determining step of the oxygen reduction mechanism.

Keywords

biofuel cell; multicopper oxidase; Tafel slope; electrochemistry; voltammetry

While the 4 e⁻ electroreduction of dioxygen to water is highly favored thermodynamically with a reversible reduction potential of 1.23 V versus the reversible hydrogen electrode (RHE), this potential is rarely realized because the reaction is kinetically slow and proceeds only at significant overpotentials (ca. -300 mV) even with the best Pt-based catalysts. The slow kinetics of the oxygen reduction reaction (ORR) is one of the major fundamental challenges

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Supporting Information Available: Synthesis and characterization of anthracene-2-methanethiol, TvLc purification procedure, electrode roughening procedure, additional characterization data, and more extensive discussion of the absence of voltammetric features under inert atmospheres. This material is available free of charge via the Internet at <http://pubs.acs.org>.

to the widespread application of fuel cells and electrocatalysis of the ORR is the subject of extensive research efforts.¹

Some multicopper oxidases, particularly certain types of laccase (Lc), reduce O₂ at significantly smaller overpotentials than Pt and have been utilized as cathode electrocatalysts in enzyme-based fuel cell applications.² Lc functions as an electrocatalyst on carbon electrodes as it adsorbs in an orientation that allows direct electron transfer (DET) from the electrode to a type 1 Cu site, subsequent internal electron transfer transports the electron to a mixed type 2/type 3 tricopper cluster where O₂ is activated and reduced.³ While poor durability and low current densities are typical, significant improvements are realized upon diazonium modification of graphite and high surface area carbon electrodes to incorporate anthracene moieties on the surface prior to the adsorption of Lc.^{4, 5} Anthracene purportedly mimics a natural substrate of the enzyme and penetrates the hydrophobic pocket near the T1 Cu site which properly orients the enzyme on the surface, facilitates electron transfer to the T1 Cu site, and reduces dissolution of enzyme off of the electrode surface. A key parameter in the characterization of electrocatalysts is the slope of a Tafel plot, however Lc modified carbon electrodes exhibit nonlinear Tafel behavior.⁴ Characterization of Lc on a better defined noble metal surface such as Au is desired to provide a potentially superior analytical platform.

On Au, Lc does not adsorb in the proper orientation for direct electrocatalysis at low overpotentials.³ Prior investigations of Lc on Au electrodes investigated a variety of strategies including adsorption of the enzyme to bare and thiol modified Au and covalent attachment to thiol modified Au.^{3, 6-10} However none of these motifs allowed observation of the direct electrocatalytic reduction of O₂ at low overpotentials. Direct electrocatalysis has been reported for a low potential (high overpotential) Lc.¹¹ Recently, it was also reported that adsorption of Lc to 50 nm Au nanoparticles allows the observation of direct electrocatalysis.¹² Herein we report that modification of a roughened Au surface with anthracene-2-methanethiol (AMT) to orient and bind Lc from *Trametes versicolor* (TvLc) enables the direct electrocatalytic reduction of O₂ at low overpotentials (see Figure 1). TvLc was chosen for study due to its intrinsically low overpotential for the ORR relative to other laccases.³ Additionally, we report successful observation of linear Tafel behavior.

A self assembled monolayer (SAM) of AMT on polished Au was characterized electrochemically and by scanning tunneling microscopy (STM). As seen in Figure 2, a hexagonal closest packed overlayer is formed by the AMT on the Au(poly) surface with nearest-neighbor spacing of *ca.* 0.4 nm. Similarly, ($\sqrt{3} \times \sqrt{3}$)R30° lattices of benzyl mercaptan and 4-biphenylmethanethiol monolayers form on Au(111) surfaces with nearest-neighbor spacing of 0.49 ± 0.01 nm.¹⁴ Based on theoretical work done by Sellers *et al.*, the methylene spacer likely promotes a vertical geometry allowing for strong intermolecular interactions between the AMT molecules.¹⁵ However, isolated anthracene moieties need to protrude from the surface in order to access the hydrophobic pocket of Lc and orient the enzyme. Indeed, we found that the ORR activity of TvLc at a polished Au electrode modified with an AMT SAM was not significant, and was comparable to prior observations made with other thiols (see Supporting Information).

In order to disrupt the SAM and create isolated anthracene moieties accessible to the enzyme we electrochemically roughened the Au surface forming rounded asperities on the order of *ca.* 100 nm in diameter as observed by atomic force microscopy (AFM) (see Supporting Information). TvLc adsorbed on the roughened Au surface modified with AMT exhibited direct electrocatalytic reduction of O₂ at potentials approaching 1 V vs. RHE (Figure 3). Use of a mixed monolayer of AMT and ethanethiol (ET) on a roughened surface (see Figure 1) increased the onset of the ORR to 1.13 V vs. RHE and yielded current densities approaching -25 $\mu\text{A}/\text{cm}^2$ in quiescent solution (Figure 3). An AMT/ET ratio of 1:3 was chosen arbitrarily for this

study, future work will seek further increases in current density and ORR onset potential that may be realized upon optimization of the thiol mixture. We attribute the improved activity to the formation of an increased number of anthracene moieties accessible to *TvLc* in the mixed monolayer.

Sparging the electrolyte with Ar leads to the disappearance of the O₂ reduction current (Figure 3). Based on the electrocatalytic current densities, we estimate that the expected current density from e⁻ transfer to the Cu sites in the absence of O₂ is too small to observe (see Supporting Information) because of the low surface concentration of *TvLc* due to the low number of accessible anthracene moieties, even with roughening and utilization of a mixed monolayer. Figure 3 also shows data obtained at a roughened electrode modified with hexadecanethiol (HDT) in place of AMT, the absence of O₂ reduction currents indicates that the anthracene moiety is required to properly orient the enzyme. Electrodes prepared with *TvLc* on roughened gold without a thiol modification, or modified with benzyl mercaptan, also did not show any significant ORR activity (see Supporting Information).

Tafel plots of the ORR at the electrodes utilizing AMT only and the AMT/ET mixture to bind *TvLc* have linear regions with slopes of *ca.* 144 mV/dec (Figure 4). The similar slope in both systems indicates that the ORR mechanism does not change as a function of the number of active *TvLc* sites on the electrode surface. *TvLc* reduces oxygen following a ping-pong type mechanism that couples the single electron oxidation of four substrate molecules to the four electron reduction to water as represented below:

where *PI* and *NI* designate the peroxy intermediate and native intermediate respectively.¹⁶ For a multistep multielectron reaction, the relationship between the transfer coefficient (α) and the reaction mechanism is described by Equation 1,

$$\alpha = \left(\frac{\gamma}{\nu} + r\beta \right) \quad (1)$$

where γ is the number of electrons transferred prior to the RDS, ν is the stoichiometric number (the number of times the step occurs per cycle of the overall reaction), r is the number of electrons transferred during the RDS, and β is the symmetry factor (usually about 0.5).¹⁷ If step I (the outer sphere single electron transfer from the electrode to the T1 Cu site) is the RDS then $\gamma = 0$ and $r = 1$ and therefore $\alpha = \beta$. If step II is the RDS then $\gamma = 4$, $\nu = 3$, and $r = 0$ and therefore $\alpha = 4/3$. If any of steps III through V are the RDS then $\gamma = 4$, $\nu = 1$, and $r = 0$ and therefore $\alpha = 4$. The value of α determined from the Tafel slope using Equation 2.¹⁷

$$\frac{dE}{d \log i} = \frac{2.3RT}{\alpha F} \quad (2)$$

The observed Tafel slope of 144 mV/dec yields $\alpha = 0.41$ which is most consistent with Step I as the RDS and $\alpha = \beta = 0.41$ which is within the normal range expected for the symmetry factor. This is consistent with the suggestion that the first electron transfer is the RDS for the oxidation of certain substrates with Lc (based on the observation that the catalytic efficiency of various laccases depends on the redox potential of the T1 Cu site).³ It also illustrates the remarkable ability of the tricopper T2/T3 site to activate the O=O bond, the typical RDS with other electrocatalysts (including Pt).¹

Linear Tafel behavior is only expected when current densities at overpotentials greater than *ca.* 118 mV are free from mass-transport effects.¹⁸ Thus, the Tafel plot of the electrode with AMT only is more linear than the electrode with the AMT/ET mixture, and the still higher geometric current density TvLc modified carbon electrodes reported by Blanford et al. do not have linear Tafel behavior.⁴ The lack of linearity may be due to mass-transport effects accompanying the higher current densities, or the heterogeneous nature of the carbon surface.

The observation of linear Tafel behavior from laccase on a modified Au surface provides an analytical platform with which to further characterize different laccase systems. While our system is suitable for the characterization of Lc at a Au surface, systems employing high surface area carbons remain at this time more practical for enzyme based fuel cells due to the accessibility of higher current densities. Optimization of laccase binding site density on Au may provide for further increases in current density in the future.

Experimental Methods

Working electrodes were of 10 mm diameter polycrystalline Au disks polished with 9.5 micron alumina powder (Buehler), sonicated in 18.2 M Ω water (Milli-pore), and wiped clean with a Kimwipe (wiping was found to improve roughening consistency). Electrochemical roughening was performed using a series of oxidation/reduction cycles as previously described except that 0.5 M KCl was employed as the electrolyte and resulted in a dark reddish brown surface.¹⁹ The roughened electrode was rinsed with water and then ethanol and then immersed in the appropriate thiol solution (0.5 mM AMT in EtOH or 0.5mM AMT + 1.5 mM ET in EtOH) for 1 h. The excess thiol was then removed by rinsing with EtOH and the electrode immersed in a TvLc solution prepared from 30 μ L of a >500 U/mL TvLc stock solution and 2970 μ L of pH 4.0 citrate/phosphate buffer. The electrode was allowed to soak for 1 h at ambient temperature prior to use (or could be soaked 24 h at 5 °C without any difference in activity). The electrode was rinsed with copious amounts of water to remove unbound Lc prior to prompt transfer to the electrochemical cell. Allowing the surface to dry results in the loss of ORR activity.

Electrochemical measurements were performed in a standard 3 electrode configuration using a CHI760C or CHI 760D potentiostat (CH Instruments). The working electrode was placed in hanging meniscus contact with the electrolyte. A Pt gauze counter electrode was separated from the working electrode by a porous glass frit and a “no leak” Ag/AgCl reference electrode (Cypress Systems) was isolated with a Luggin capillary. The headspace of the cell was closed and either Ar or O₂ atmospheres were employed. The electrolyte consisted of 0.1 M citric acid adjusted to pH 4.00 with 0.2 M Na₂HPO₄.

STM measurements were carried out using a Nanoscope III E (Digital Instruments). The gold substrate was clamped to the stage using a Teflon cell. Pt-Ir STM tips (0.25 mm in diameter) were purchased from Veeco Instruments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Schematic representation of Lc from *Trametes versicolor* properly oriented for electrocatalytic reduction of O₂ at an electrochemically roughened Au surface modified with a mixture of anthracene-2-methanethiol (AMT) and ethanethiol (ET). The AMT is shown in the hydrophobic pocket where it facilitates electron transfer from the electrode surface to the T1 Cu site. After an internal electron transfer, the 4 e⁻ reduction of O₂ to water occurs at the T2/T3 tricopper cluster near the center of the enzyme.¹³

Figure 2. STM micrograph of hexagonal closest packed AMT monolayer formed on a polycrystalline Au surface. Nearest-neighbor spacing is *ca.* 0.4 nm within each domain. ($V_{\text{bias}} = 150 \text{ mV}$, $i_t = 2.00 \text{ nA}$)

Figure 3.

Cyclic voltammograms showing the electrocatalytic activity of *TvLc* adsorbed on a roughened Au electrode modified with: an AMT/ET mixture (red), AMT only (blue), and HDT (green) in O_2 saturated electrolyte; and Lc on the AMT/ET modified electrode in Ar saturated solution (black). Data recorded at 5 mV/s in 0.1 M citric acid buffer adjusted to pH 4.00 with 0.2 M Na_2HPO_4 .

Figure 4.

Tafel plots of the O_2 reduction activity of TvLc adsorbed on roughened Au electrodes modified with AMT (blue) and an AMT/ET mixture (red) in O_2 saturated pH 4.00 citrate-phosphate buffer; and linear fits equivalent to Tafel slopes of 144 mV/dec (short dashes) and 145 mV/dec (long dashes). Data recorded during a 5 mV/s cathodic potential scan.